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Microsite Factors and Spore Dispersal Limit Obligate Mycorrhizal Fern Distribution: Habitat Islands of *Botrychium pumicola* (Ophioglossaceae)

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ABSTRACT.—We investigated the influence of microsite and establishment factors on the distribution of *Botrychium pumicola* Coville ex Underw. (Ophioglossaceae), an obligate mycorrhizal fern restricted to specialized volcanic habitats, predominately pumice, in the Cascade Range of central and southern Oregon and northern California. The fragmented distribution of this threatened species and its absence from suitable habitat is of conservation concern. At eight subalpine and one alpine site, plots with and without *B. pumicola* were compared for biotic and abiotic variables. *Botrychium pumicola* populations occurred with diverse perennial plants. Nearest neighbor analysis showed a clumped dispersion of *B. pumicola* with associated plants. *Botrychium pumicola* spores were found in soils of seven plots with and two without *B. pumicola*. In plots with *B. pumicola*, soil potassium was higher while soil density and soil temperatures were lower than in plots without *B. pumicola*. Airborne spores, collected in spore traps, were dispersed up to 10 m from source plants. Microsite factors (soil nutrients, soil density, nearest neighbor dispersion) and dispersal limitations are likely key factors influencing the distribution of *Botrychium pumicola*. Narrow spore germination requirements combined with dispersal limitations may influence population expansion of obligate mycorrhizal ferns such as *B. pumicola*.

KEY WORDS.—*Botrychium*, endemism, mycorrhizal ferns, Ophioglossaceae, pumice, pumice moonwort, rare ferns, spore bank, spore dispersal

Causes of rarity in herbaceous plants are complex with multiple components (Stebbins, 1980; Kruckeberg and Rabinowitz, 1985; Fiedler, 1986). Proximate factors leading to rarity include intrinsic fitness (limited propagules, slow growth), requirement for mutualists (mycorrhizal symbionts, dispersal agents, pollinators), plant-soil interactions (inadequate soil components), plant-plant interactions (competition), meteorological factors (wind, precipitation, temperature), disturbance (changes in hydrology or nutrients, animal and human effects), and climate change (increased temperatures, changes in snowpack and moisture). The relative importance of these components has implications for conservation and rare species management. Factors such as intrinsic fitness and meteorological conditions are not subject to management; others such as disturbance, requirement for mutualists, and some of the effects of climate change may be ameliorated by management activities.

In ferns, the apparent ease of spore dispersal and the development of spore banks imply that microsite factors play a greater role than dispersal limitations

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in determining fern distribution (Tryon, 1970; Dyer and Lindsay, 1992; Kato, 1993). Habitat specificity is often implicated as the critical factor in fern distribution (Richard *et al.*, 2000; Guo *et al.*, 2003; Jones *et al.*, 2006). However, for some fern species, stochastic factors, including limited spore dispersal combined with habitat specificity influenced fern establishment (Peck *et al.*, 1990; Karst *et al.*, 2005; Wild and Gagnon, 2005). The distribution of obligate mycorrhizal ferns with narrow spore germination requirements (photoinhibition and arbuscular mycorrhizal inoculation) may be more influenced by microsite and establishment factors than other fern species. Conditions near established populations (available arbuscular mycorrhizal inoculum, similar soil nutrients and microclimate) are more likely to offer suitable habitat than more distant sites (Johnson-Groh and Lee, 2002; Karst *et al.*, 2005).

Botrychium pumicola Coville ex Underw. (Ophioglossaceae), pumice moonwort, is endemic to the Cascade Range of central to southern Oregon and northern California, and to the pumice plume areas of central Oregon (Farrar, 2012). A former Federal Category 1 Candidate for listing as Threatened and a former Federal Species of Concern, *B. pumicola* is listed as Threatened by the State of Oregon (USFWS, 1980; Lewis, 1996; Kagan *et al.*, 2010; ODA, 2012). It is on the U.S. Forest Service Region 6 Sensitive Species List, and is a U.S. Bureau of Land Management Special Status Plant and a California Special Status Plant (Hopkins *et al.*, 2001; ISSSSP, 2012; CDFG, 2013). First collected by Frederick V. Coville from Llao Rock in what would become Crater Lake National Park, *B. pumicola* grows in pumice fields, on pumice scree, and on volcanic rubble (Underwood, 1900; Wagner and Wagner, 1993; Hopkins *et al.*, 2001; Roe-Andersen, 2010). In spite of the presence of ripe sporangia with dispersed spores, this species is absent from adjacent pumice fields and other volcanic habitats that appear suitable (Hopkins *et al.*, 2001; Roe-Andersen, 2010).

Pumice soils derived from Mazama and Newberry plumes are characterized by low fertility, little soil profile development, and high porosity (Youngberg and Dyrness, 1964). In exposed pumice fields and open volcanic habitats, microtopographic features trap wind-blown materials and relief-driven moisture (rain and snow), creating microsites and favorable conditions for fern life cycle development (Körner, 1999; Titus *et al.*, 2007). Several flowering plant species including *Arnica viscosa* A. Gray (Asteraceae), *Boechera horizontalis* (Greene) Windham and Al-Shehbaz (formerly *Arabis suffrutescens* var. *horizontalis*, Brassicaceae), and *Astragalus peckii* Piper (Fabaceae) are endemic to pumice and volcanic substrates (Wolf and Denford, 1984; Windham and Al-Shehbaz. 2007; Martin and Meinke, 2012).

The goals of this study were to examine microsite factors and dispersal patterns that might affect the distribution of alpine and subalpine populations of *B. pumicola*. Specifically the objectives were to determine if environmental variables differed in areas inhabited by *B. pumicola* and in adjacent areas without *B. pumicola* and to evaluate dispersal capability. Our hypotheses are that: (1) adjacent plots with and without *B. pumicola* differ with regard to soil nutrients, soil texture, and site characteristics; (2) adjacent plots with and

without *B. pumicola* differ with regard to density of associated plant species, and *B. pumicola* spore bank; (3) *B. pumicola* dispersion is clumped; and (4) *B. pumicola* spore dispersal is predominantly local.

Materials and Methods

Plant natural history.—Leaves of *Botrychium pumicola* emerge after spring snowmelt, with peak emergence mid-July to mid-August (Hopkins *et al.*, 2001; Roe-Andersen, 2010). Leaves reach heights of 1–6 cm above the soil surface, with 7–10 cm of the frond below ground. The stem with leaf primordia at the apex remains underground. The single leaf that matures each year is divided into a sterile trophophore and fertile sporophore. Reproduction occurs through spores, gemmae and intragametophytic selfing (Camacho, 1996; Camacho and Liston, 2001). Spores require a period of darkness for germination (Whittier, 1973; Johnson-Groh, 2002). Photoinhibition of spore germination ensures below-ground germination where the likelihood of sufficient moisture and proximity to mycorrhizal symbionts improve chances for colonization (Whittier, 2006).

Botrychium species form obligate symbioses with arbuscular mycorrhizal (AM) fungi, predominately *Glomus* spp. (Kovacs *et al.*, 2007; Winther and Friedman, 2007). Sporophytes of other *Botrychium* species remain underground for several years, relying on mycorrhizal partners to obtain fixed carbon from neighboring plants through an AM fungal network (Johnson-Groh, 1998; Winther and Friedman, 2007).

Study sites.—Populations were studied at eight subalpine sites and one alpine site: Broken Top (BR), Mt. Bachelor (BA), and the Dome (DO) in the Deschutes National Forest (DNF); Llao Rock with two populations, Llao East (LE) and Llao West (LW), Grotto Cove (GR), Skell Head (SK), Cloud Cap with two populations, Cloud Cap North (CN) and Cloud Cap South (CS), and Dutton Ridge (DU) in Crater Lake National Park (CLNP), Oregon; and Shastina Cone (SH) in the Shasta-Trinity National Forest (STNF), California (Fig. 1, Table 1; Roe-Andersen, 2010). Montane populations at lower elevations were not included. All sites receive heavy snow accumulation (up to 3 m) with average minimum temperatures below freezing for eight months of the year (Hopkins *et al.*, 2001; WRCDC, 2012). Average summer precipitation is low (3.1 cm total) and daily temperatures fluctuate widely (1.1–20.9°C). Plants are exposed to intense heat, cold, ultraviolet radiation, and strong winds.

Sites in CLNP were located in exposed, wind-swept pumice fields surrounding the caldera (Fig. 1B). Substrates consisted of volcanic "popcorn" pumice and mixed volcanic gravel from the Mt. Mazama pumice outfall c. 7700 years ago (Fig. 2A; Klimasauskas *et al.*, 2002). In the DNF, the subalpine site (DO) in Newberry National Volcanic Monument, is a volcanic cinder cone approximately 11,000 years old, covered with 0.5–1 m of Newberry "popcorn" pumice from the Big Obsidian Flow eruption c. 1300 years ago (Sherrod *et al.*, 1997). On the southeast flank of Broken Top Mountain, the BR site consisted of eroded andesite, dacite and rhyodacite from 100,000-year-old Broken Top

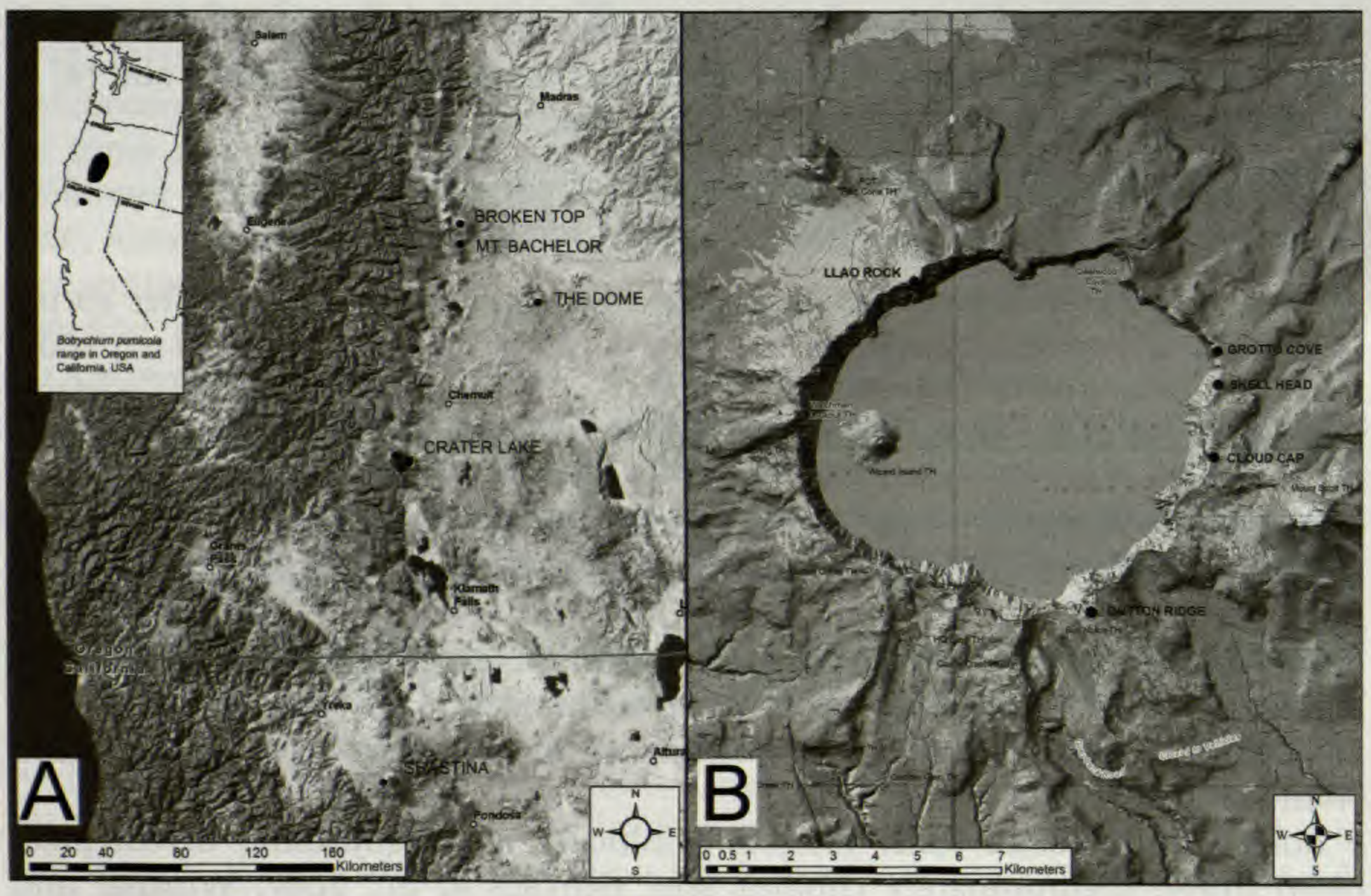

FIG. 1. *Botrychium pumicola* study sites. (A) The Cascade Range of central and southern Oregon and northern California. (B) Crater Lake National Park, Oregon.

eruptions, mixed with pumice and volcanic gravel from the South Sister eruption c. 2000 years ago (Scott *et al.*, 2003; Fig. 2B). The alpine site (BA) on the summit of Mt. Bachelor was in open, windswept volcanic rubble from Mt. Bachelor summit eruptions c. 12,000 years ago, and small amounts of pumice from the Mt. Mazama tephra plume c. 7700 years ago (Scott, 1990). The SH site was located on an exposed, windy, southwest flank near the upper end of Diller Canyon and consisted of andesitic boulder fields creating rock shelters for *B. pumicola* (Fig. 2C). This population was rediscovered 13 Jul 2009 (Eric

TABLE 1. *Botrychium pumicola* study sites arranged by elevation in the Cascade Range of central and southern Oregon and northern California. CLNP, Crater Lake National Park; DNF, Deschutes National Forest; STNF, Shasta-Trinity National Forest.

Study Site	Location	Size (ha)	Elev (m) asl	Aspect	Slope	*B. pumicola* count
Grotto Cove	CLNP	0.03	2100	12°	3–10°	13
Skell Head	CLNP	0.03	2180	250°	7.5°	37
The Dome	DNF	0.05	2190	18°	12°	75
Broken Top	DNF	0.10	2330	82°–140°	15°	636
Cloud Cap	CLNP	0.43	2400	185°–250°	19–27°	137
Llao Rock	CLNP	0.45	2440	110°, 250–290°	0–19°	>1000
Dutton Ridge	CLNP	0.03	2460	190°	9°	1
Shastina Cone	STNF	0.03	2750	220°	15–17°	6
Mt. Bachelor	DNF	0.10	2760	129°–172°	15°	372

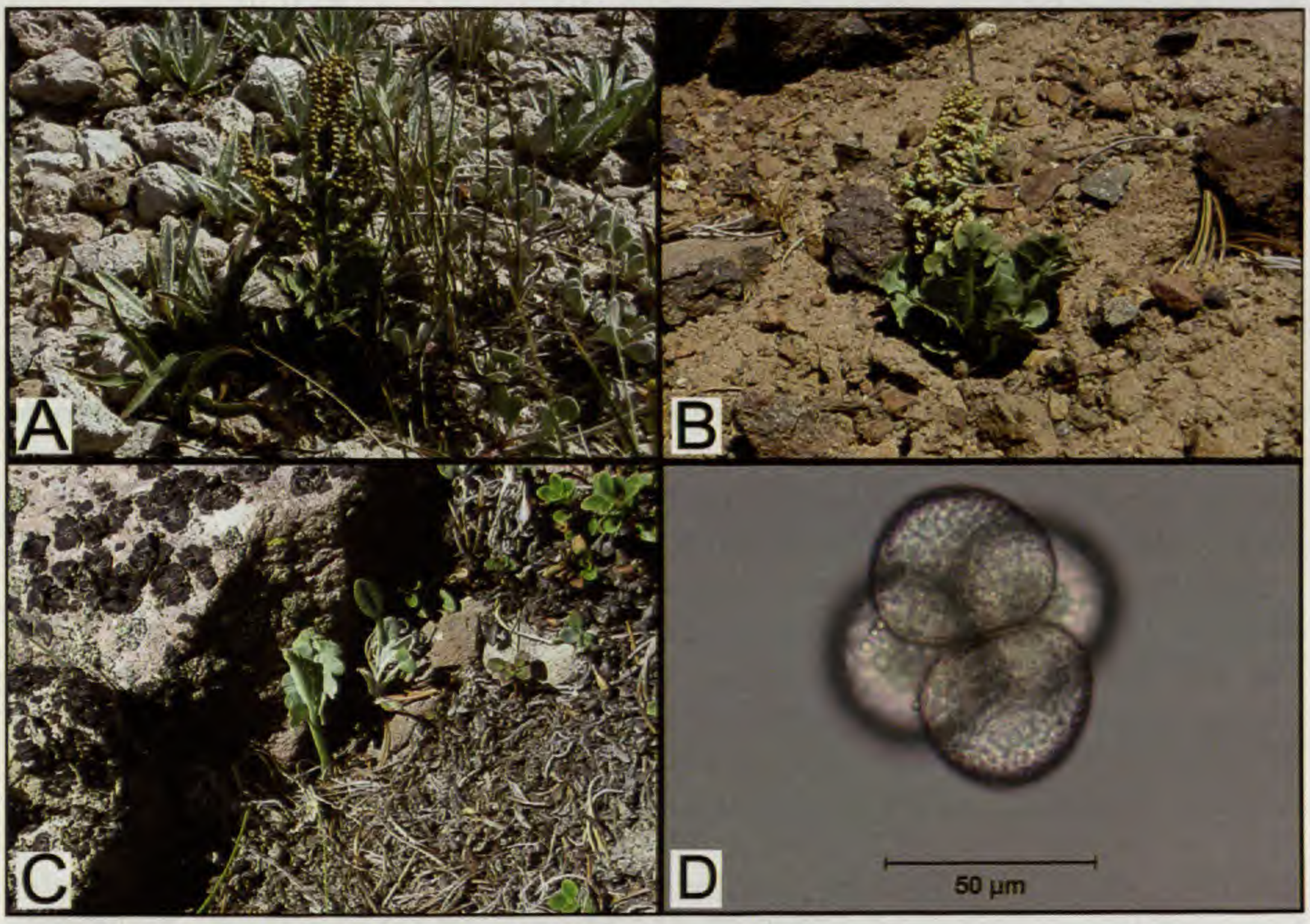

FIG. 2. *Botrychium pumicola* in the Cascade Range of central and southern Oregon and northern California. A–C, *B. pumicola* habitat. (A) Classic subalpine "popcorn" pumice, Llao Rock, Crater Lake National Park (CLNP). (B) Clay soil and few neighboring plants, Broken Top, Deschutes National Forest. (C) Andesitic rock shelter, Shastina Cone, Shasta-Trinity National Forest. (D) *B. pumicola* spore tetrad from soil sieving, Grotto Cove, CLNP.

White, U.S. Forest Service, pers. comm.). Shastina Cone formed 9700–9400 years ago as a subsidiary andesite cone on the west flank of Mt. Shasta (Christiansen, 1990).

Sampling design.—At each site, plots with *B. pumicola* were paired with adjacent plots without *B. pumicola*, but similar in slope, aspect, elevation, and vegetation. Because environmental variables at fine spatial scales influence the distribution and abundance of ferns (Karst *et al.*, 2005), paired sites were located within 5–10 m of plots where *B. pumicola* occurred. Paired sites were chosen in areas where no *B. pumicola* had been observed for the past three consecutive years. At each site, 10 1-m^2 plots (5 with *B. pumicola*) were chosen at random and sampled for environmental variables (Elzinga *et al.*, 1998). At LE and LW with larger populations, 10 plots with *B. pumicola* and 10 without were sampled.

Populations were mapped using a Garmin GPSmap 60CSx (Garmin International, Olathe, Kansas). Maps were created in ArcGIS v. 9.3 (ESRI, Redlands, California). Elevation was determined with the GPS unit and field checked with USGS 7-minute topographic maps (Crater Lake East, Crater Lake West, Broken Top, Mt. Bachelor, East Lake, and Mt. Shasta topographic

quadrangles). Slope was determined with a Suunto clinometer (Suunto Inc., Vantaa, Finland) and aspect with a Silva Ranger compass (Johnson Outdoors Inc., Racine, Wisconsin). In each 1-m^2 plot, all plant species were identified and stems counted.

Data collection began 22 Jun 2009 and ended 28 Aug 2009 when most *B. pumicola* plants had senesced. To minimize researcher impact on fragile pumice substrates, workers wore light-weight, soft-soled "pumice shoes" and stooped beside plots during data collection. Sampling was carried out in relation to *B. pumicola* phenology, beginning at the lowest elevation site and moving up in elevation so that all populations were sampled during the spore-release stage (Johnson-Groh and Lee, 2002). Sampling was conducted once during the growing season which has been found to be sufficient in subalpine sites to detect most *Botrychium* plants (Lesica and Ahlenslager, 1996).

Botrychium pumicola spore dispersal.—Spores were isolated from soil samples by centrifugation (Mason and Farrar, 1989; Brundrett *et al.*, 1996; Fig. 2D). Johnson-Groh *et al.* (2002) sampled soils for *Botrychium* gametophytes to a depth of 10 cm, and because density of fern spores was higher in the upper 10 cm of soil (del Ramirez-Trejo *et al.*, 2004), soil samples for spore isolation were taken to a depth of 10 cm, and stored at 15–21°C for 90–120 days until processing. For each sample, 250 mL of soil was mixed with 250 mL distilled water and soaked for 30 min. Wetted soil was decanted through Tyler Standard Screen soil sieves (W.S. Tyler, Mentor, Ohio) and flushed with tap water. Number 200 (75 μm opening) and 325 (43 μm opening) sieves plus the catch basin, were used to capture *B. pumicola* spores (70 μm tetrad). Sievings were placed in 50-ml centrifuge tubes and spun in a Sorvall RC 5C Plus Centrifuge (Thermo Fisher Scientific Inc., Waltham, Massachusetts) for 5 min at 2000 RPM. Supernatant was discarded, and the pellet resuspended in 50% sucrose and centrifuged (3 min, 2000 RPM). Floating material was transferred to microscope slides and examined using a Leica DMLB compound microscope. Spores were photographed using a SPOT Insight digital camera (Diagnostic Instruments, Inc., Sterling Heights, Michigan). Although *Botrychium lanceolatum* (Gmelin) Angström subsp. *lanceolatum* was present at LE, and *B. multifidum* Gmelin and *B. simplex* Hitchcock occurred 4 km from DO, these species release spores individually, while *B. pumicola* spores usually remain in tetrads (Wagner, 1998; Farrar, 2011). To ensure accurate species identification, only spores in tetrads were counted to determine spore density of *B. pumicola*. Spore counts were binned as 0, 1 (1–5), 2 (6–20), 3 (>20).

Airborne spores were trapped during peak spore release (10 August through 8 September) at SK, DU, and north of LE (LNE), a site with 300 *B. pumicola*. Spore traps were constructed by placing 5 cm of double-sided cellophane tape on glass microscope slides and either laying them on the ground or attaching slides vertically to a metal wire (Peck *et al.*, 1990). At DU, spore traps were installed in a symmetrical array from an isolated *B. pumicola*: two horizontal spore traps at 0.5 cm, four vertical traps at 10, 20, 30 cm, and 1, 2, and 3 m. In addition four vertical traps were placed on a north-south axis 5 and 10 m from the eastern edge of the population in line with prevailing winds. At SK, spore

traps were installed in a symmetrical array from an isolated clump of two *B. pumicola* individuals: four horizontal traps at 0.5 cm, four vertical traps radiating out from two *B. pumicola* plants at 10, 20, and 30 cm and four vertical traps at 1, 2, and 3 m. In addition five vertical traps were placed on a north-south axis 5, 10 and 20 m from the southeast edge of the population and four vertical traps 5 m from the northern edge of the population in line with prevailing winds. At LNE, five vertical spore traps were installed 1, 5, 10 and 20 m from southern edge of the population and 1, 5, and 10 m from western edge of the population in line with prevailing winds. Spore traps were recovered after two weeks, examined dry with light microscopy, and spore tetrads counted.

Soil analyses.—Soil temperature readings were taken in each 1-m^2 plot 10 cm below the soil surface using a Taylor Commercial digital probe thermometer model 9842 (Taylor Precision Products, Las Cruces, New Mexico; accuracy range ± 0.6° C). To avoid damaging *B. pumicola* plants, temperature readings were taken 2–3 cm from a *B. pumicola* plant in plots with *B. pumicola* and from another plant species in plots without *B. pumicola*.

Soil samples (500-mL) were taken at depths of 10–12 cm from one randomly selected plot with *B. pumicola* and one plot without *B. pumicola* for each population except for SH which was not sampled. The sample at DU was taken 10 cm from the *B. pumicola* individual, to avoid removing any potential germlings from this small population. Samples were stored at 0° C and transported on ice to the Soil Analytical Laboratory at Oregon State University, Corvallis, USA, for testing of soil moisture, organic matter, pH, P, K, Mg, Na, Ca, NO_3, NH_4, soil density and particle size using the methods of Horneck *et al.* (1989).

Data analyses.—Environmental variables were divided into abiotic factors including soil pH, soil nutrients, soil textural properties and site characteristics (slope, aspect and elevation), and biotic factors including *B. pumicola* phenology, other plant species, and nearest neighbors (*B. pumicola* and closest associated plant species). The 95% confidence interval was used as the level of significance for all analyses. Data were analyzed with a Wilcoxon matched pairs test using Statistica v.7 software (StatSoft Inc., Tulsa, Oklahoma) to determine if plots with and without *B. pumicola* differed.

A multivariate analysis of environmental variables was conducted using PC-ORD v. 5 software (MJM Software, Gleneden Beach, Oregon). Using Non-Metric Multidimensional scaling (NMS), ordinations were run for each group of environmental variables to demonstrate any separation between plots with and without *B. pumicola*. All ordinations used the Sørensen (Bray-Curtis) distance measure. McCune and Mefford (2006) recommend removing outliers due to their potential to skew data, so an outlier analysis was conducted for all environmental variables, outliers with high calcium were removed, and the NMS test was re-run. Differences were analyzed using Multi-Response Permutation Procedures (MRPP) in PC-ORD to test the hypothesis of no difference between two or more groups of environmental variables. As with NMS, outliers with high calcium were removed, and the test rerun.

TABLE 2. Soil temperature (mean) of plots with and without *Botrychium pumicola* and test for differences by Wilcoxon Matched Pairs test for alpine and subalpine populations in the Cascade Range of central and southern Oregon.

	Mean soil temp °C	
Population	*B. pumicola present*	*B. pumicola absent*
Llao West	17.87	20.81
Llao East	10.25	14.35
Broken Top	20.58	20.32
Dome	22.32	23.92
Grotto Cove	24.30	20.68
Dutton Ridge	15.60	15.70
Skell Head	19.10	18.68
Mt. Bachelor	13.74	15.08
Mean (sd)	17.97 (4.48)	18.69 (3.37)
P (Wilcoxon)	0.006[a]	

[a] significant at $P < .05$

Associated plant species data were modified by removing species occurring in fewer than 5% of plots. In order to minimize the effect of disproportionately large numbers in the plant associate data set, a relativization was applied. Species that occurred in only one population (singletons) were removed from the data set. Species-area curves were constructed in PC-ORD 5.0 using Sørensen distance measure (McCune and Grace, 2002).

Nearest neighbor analysis was conducted to determine the spatial dispersion between each *B. pumicola* individual and the nearest conspecific or other plant species. A Nearest Neighbor Index (NNI) was calculated (Clark and Evans, 1955).Values approaching zero indicate a clumped distribution, values near one indicate a random distribution, and a value near 2.15 indicates a uniform distribution.

Aspect measurements for each population were binned into one of eight cardinal directions: North (N) 336–25°; North East (NE) 26–70°; East (E) 71–110°; South East (SE) 111–160°; South (S) 161–200°; South West (SW) 201–250°; West (W) 251–290°; North West (NW) 291–335°. Binned aspects were compared with a Chi-square Goodness of Fit Test (Minitab v.15, Minitab, State College, Pennsylvania).

RESULTS

Study sites ranged in elevation from 2100 to 2760 m. Aspect ranged from 12° to 290° on slopes from relatively flat to 27° (Table 1). *Botrychium pumicola* occurred on binned aspects: west (five populations); south and southwest (four populations each); southeast (two populations); north, northeast and east (one population each). No populations occurred on binned northwest aspects (Chi-square Goodness of Fit, $P = 0.045$).

Abiotic factors.—Soil temperatures were lower in plots with *B. pumicola* (Wilcoxon Matched Pairs, $P = 0.006$; Table 2). Soil textural properties of

TABLE 3. Soil textural properties in paired plots with *B. pumicola* present (P) or absent (A) compared by Wilcoxon Matched Pairs test for alpine and subalpine populations in the Cascade Range of central and southern Oregon.

Population	Density (g/L)		OM %		H_2O %		Gravel %		Sand %		Silt %		Clay %	
B. pumicola	P	A	P	A	P	A	P	A	P	A	P	A	P	A
LW	371.3	505.1	2.8	1.5	4.3	1.0	23.7	17.9	91.3	93.8	7.5	3.8	1.3	2.5
LE	371.7	559.6	2.5	0.9	11.9	8.1	20.0	35.4	91.3	97.5	5.0	1.3	3.8	1.3
BR	405.6	508.4	2.8	2.7	2.5	1.9	34.7	49.1	83.8	82.5	12.5	13.8	3.8	3.8
DO	342.5	404.5	2.3	2.8	5.5	9.2	56.0	67.0	85.0	81.3	12.5	15.0	2.5	3.8
GR	532.4	460.4	1.0	0.9	1.0	1.1	25.1	25.3	92.5	97.5	3.8	1.3	3.8	1.3
DU	499.7	542.1	1.8	1.5	4.3	7.5	13.7	31.3	92.5	92.5	5.0	6.3	2.5	1.3
CS	465.7	558.8	1.8	1.7	1.9	1.4	36.9	37.4	90.0	92.5	7.5	3.8	2.5	3.8
SK	357.0	483.6	1.0	1.0	5.1	9.4	21.7	23.0	96.3	90.0	2.5	6.3	1.3	3.8
BA	494.7	493.7	1.9	1.9	9.0	11.3	20.4	20.3	86.3	82.5	10.0	10.0	3.8	3.8
Mean	426.7	501.8	2.0	1.7	5.1	5.6	28.0	34.1	89.9	90.0	7.4	6.8	2.8	2.8
sd	71.7	49.8	0.7	0.7	3.5	4.2	12.8	15.7	4.1	6.4	3.7	5.1	1.0	1.2
P	0.03 [a]		0.07		0.68		0.05		0.89		0.48		0.80	

[a] Significant at $P < .05$

percent sand, silt, or clay did not differ between paired plots with and without *B. pumicola*; soil density was lower in plots with *B. pumicola* in seven out of nine pairs (Wilcoxon Matched Pairs, $P = 0.032$; Table 3). Paired plots did not differ in pH, phosphate, magnesium, sodium, calcium, nitrate, ammonium (Table 4), or in percent organic matter (Table 3). Potassium was higher in plots with *B. pumicola* (Wilcoxon Matched Pairs, $P = 0.024$; Table 4), although similar values of K were found in some plots without *B. pumicola*.

NMS ordination of all abiotic factors showed a high degree of overlap between plots with and without *B. pumicola*. No differences in abiotic factors were observed using Multi-Response Permutation Procedure (MRPP) for all populations ($P = 0.180$). When outliers (values >2 standard deviations from the mean) were removed, differences in abiotic factors were significant (MRPP, $P = 0.049$; Fig. 3).

Biotic factors.—Thirty-five plant species occurred in plots with *B. pumicola* and 32 in plots without *B. pumicola* (Table 5). The most abundant species was *Raillardella argentea*, and the most frequent were *Carex breweri* and *Lupinus lepidus*. No plant species, except *B. pumicola*, was present at all sites. Species-area curves indicated that plots with *B. pumicola* were similar in species richness of associated plants (first order jackknife estimate, 44.9) to plots without *B. pumicola* (first order jackknife estimate, 42.5). For an accumulated eight sites, species-area curves of plots with and without *B. pumicola* had 32 associated species. The density of associated plants showed a high range of variability in plots with and without *B. pumicola* (Table 5).

NMS ordination of all plant associates showed differences between plots with and without *B. pumicola* (MRPP, $P = 0.015$). When abundance values were relativized to minimize the effect of plots with disproportionately large

TABLE 4. Soil pH and soil nutrients in paired plots with *B. pumicola* present (P) or absent (A) compared by Wilcoxon Matched Pairs test for alpine and subalpine populations in the Cascade Range of central and southern Oregon.

Population	pH		P (mg/kg)		K (mg/kg)		Mg (mg/kg)		Na (mg/kg)		Ca (mg/kg)		NO_3 (mg/kg)		NH_4 (mg/kg)	
Botrychium pumicola	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A
LW	6.3	6.5	30	27	88	63	47	54	21	15	405	217	1.7	0.8	2	1.4
LE	6.5	6.5	20	35	88	47	59	31	19	15	513	117	1.3	0.9	1.6	1.0
BR	6.3	5.7	40	50	82	39	129	23	20	13	461	45	1.3	1.3	4.9	1.2
DO	6.3	6.3	10	12	119	98	136	145	27	22	597	589	3.1	3.2	2.3	1.7
GR	6.2	6.5	48	43	52	47	27	26	29	19	95	112	0.3	0.8	0.9	1.0
DU	6.3	6.4	45	39	71	76	31	38	13	18	167	171	0.09	1.3	1.2	1.4
CS	6.6	6.5	12	14	91	90	111	122	14	19	329	372	0.7	1.0	1.1	1.2
SK	6.5	6.4	30	31	63	59	43	38	28	19	176	157	0.4	0.7	1.0	1.0
BA	6.5	6.4	19	15	107	82	194	488	24	20	1230	2010	0.6	0.9	1.5	1.4
Mean	6.4	6.4	28.2	29.6	84.6	66.8	86.3	107.2	21.7	17.8	441.0	421.0	1.1	1.2	1.8	1.3
sd	0.1	0.3	14.0	13.6	20.8	20.8	58.4	149.4	5.8	2.9	341.0	618.0	0.9	0.8	1.2	0.2
P	0.87		0.95		0.02 [a]		0.59		0.09		0.59		0.40		0.12	

[a] Significant at $P < 0.05$

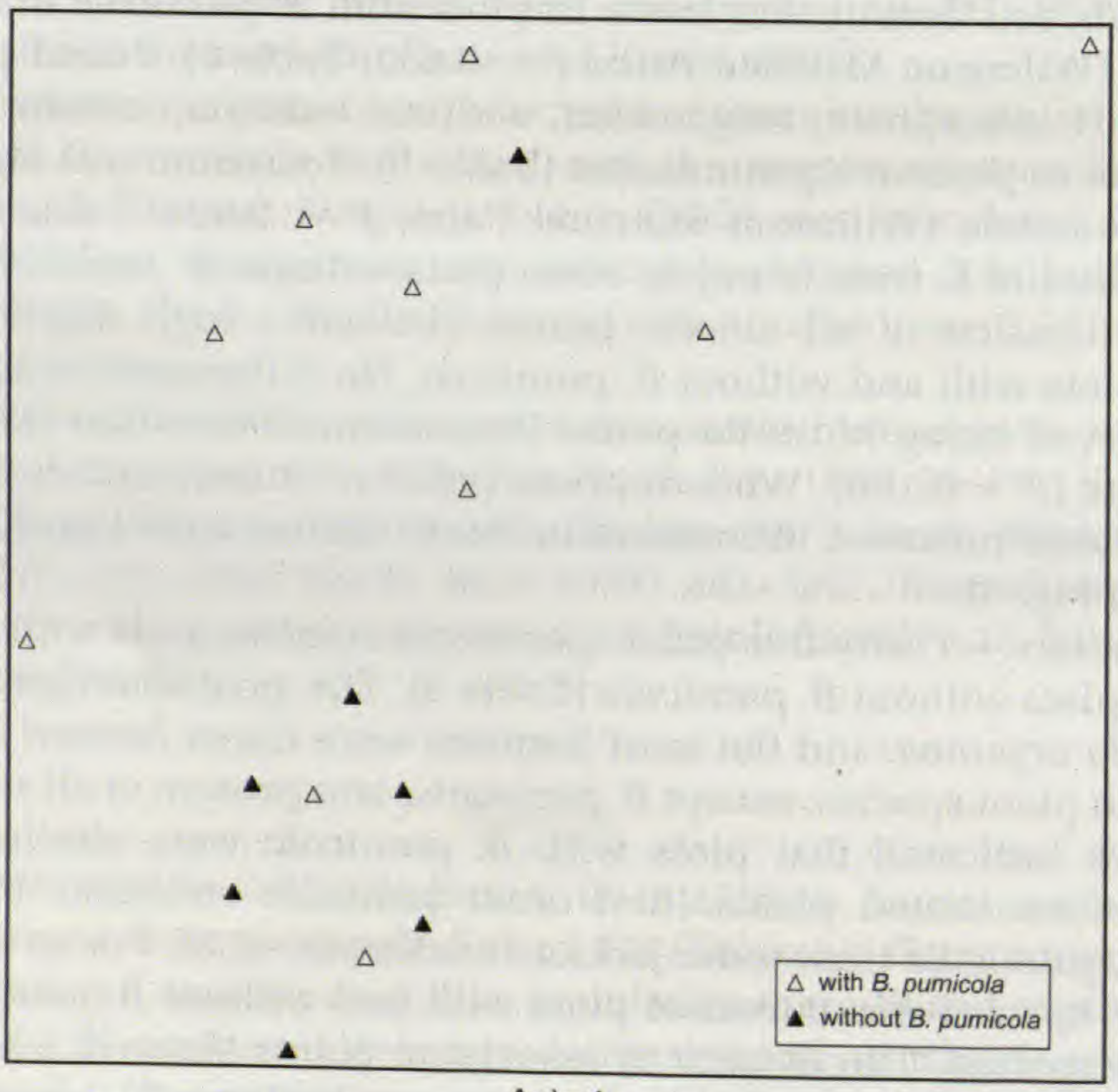

FIG. 3. NMS ordination for *Botrychium pumicola* populations in the Cascade Range of central and southern Oregon. All abiotic factors ordinated by site; outliers with high calcium removed; Δ = with *B. pumicola*, ▲ = without *B. pumicola*.

numbers, plots with and without *B. pumicola* still differed (MRPP, $P = 0.007$). When associated plant species occurring in fewer than 5% of plots were removed, plots with and without *B. pumicola* differed in community composition (MRPP, $P = 0.018$; Fig. 4). NMS ordination of all plant associates per m^2 with singletons removed showed no differences between sites with and without *B. pumicola* (MRPP, $P = 0.875$). NMS ordination of abiotic factors plus plant associates with singletons removed showed no difference between plots with and without *B. pumicola* (MRPP, $P = 0.875$).

Nearest-neighbor distances between *B. pumicola* conspecifics ranged from 9.1 to 71.6 cm with clumped and random dispersions (Table 6). In four populations, NNIs were below 1.00 indicating clumping; in five populations, NNIs of 1.10–1.33 indicated a random distribution. Nearest neighbor distances between *B. pumicola* individuals and the nearest associated plant species ranged from 1.6 to 15.4 cm (Table 6). Nearest neighbor indices between *B. pumicola* individuals and the nearest other plant species showed a clumped dispersion (NNI < 1.00) in all populations except at BR which had an NNI of 2.04 indicating a uniform dispersion (Table 6).

Spore tetrads of *Botrychium pumicola* were found in soil samples from plots with *B. pumicola* (Fig. 2D) at LE (20); LW (1); SK (4); CN (1); CS (2); BR and BA (1 each) and in plots without *B. pumicola* at GR (3); BA (1). No *B. pumicola* spores were found at DU. One single spore (not in tetrad) was found during sampling of BR (*B. pumicola* present) soils. Spore tetrads were found on spore traps at distances up to 10 m. At SK, spores lodged on slides 10 m from the source plant with most spores lodging within 30 cm. At DU spores lodged on slides within 1 m from the source plant. At LNE spores lodged on slides 10 m from the source plant with most spores lodging within 5 m (Fig. 5).

Discussion

Microsite factors.—In paired plots with and without *B. pumicola*, K levels were higher in the plots with *B. pumicola*. Potassium is essential for plant growth, development and regulation of stomata, with the role of K in photosynthesis becoming more important at higher CO_2 levels and light intensity (Larcher, 2002; Cakmak, 2005). A higher K level may help *B. pumicola* regulate water loss in habitats where plants are subject to low summer precipitation, intense UV radiation, temperature extremes, and desiccating winds. Potassium is essential for spore germination in ferns (Miller and Wagner, 1987). Lower K levels may decrease gametophyte success in habitats that appear suitable for *B. pumicola*.

Although several *Botrychium* species are associated with calcareous habitats (Cayouette and Farrar, 2009; Zika *et al.*, 2002), *B. pumicola* is not a calcicole, and calcium did not differ between sites with and without *B. pumicola*.

Soil density was lower in plots with *B. pumicola*. Greater porosity may enable *B. pumicola* spores to move down the soil column. This may assist spores in finding the darkness necessary for germination and the AM fungal partners for continued growth (Johnson-Groh, 2002; Whittier, 2006).

TABLE 5. Plant density (stems per m^2) at alpine and subalpine sites with *Botrychium pumicola* present (P) and absent (A) in the Cascade Range of central and southern Oregon and northern California.

Botrychium pumicola (P/A)	P	A	P	A	P	A	P	A	P	A	P	A	P	P	A	P	A	P
Plant Associate	LW	LW	LE	LE	BR	BR	DO	DO	GR	GR	DU	DU	CN	SK	SK	BA	BA	SH
Achillea millefolium					34.4		9	11.2										
Achnatherum occidentale									5.2	6.6	20	20	0.2	8	0.8			
Agoseris sp.	20.1	8.8	6.5	4.7			1.2	2.2		2.4			0.4		0.6			
Antennaria alpina var. *media*	7.3		4.4	0.3										12.8	13.8			
Antennaria geyeri									3.6									
Antennaria rosea		10.6								2.4			15.8					
Arabis divaricarpa																0.2		
Arabis lyallii			0.2							0.2								
Arabis platysperma										0.4								
Boechera horizontalis	0.2	0.1											0.2					
Calyptridium umbellatum					0.2				1	1				3.6	5.2	1.4	0.4	
Castilleja applegatei							12	6.8										
Castilleja arachnoidea	8.5	3	4.3	0.6	0.4	4.4												
Carex breweri	24	20.7	40.2	35.7	1.8	24.4			14.4	6.4	10	12	10.4	6.8	9.2	15.2	29.2	
Carex inops	12.4	2.1																
Collinsia parviflora							3.6	0.2										
Elymus elymoides	2.5	8.9	7	14.4	1.8	1.4			3.2	3.2	0	0	7.6	4.8	6.2			
Ericameria nauseosa									0.6									
Erigeron compositus									1.4				1			0.2	0.8	1
Erigeron elegantalus		0.9								0.6			24.4	17.4	25			
Eriogonum ovalifolium	0.1		1.1	5.4					0.2				3.6		1.2			
Eriogonum pyrolifolium						0.6												
Eriogonum umbellatum	4.8	7.7	5	8.9	0.4	4	2.4	0.6	1	0.8			2	3.2	2			
Eriophyllum lanatum										0.2				0.6	1.2			
Festuca idahoensis													2.2					
Gayophytum diffusum							3.8	0.8										
Holodiscus microphyllus																		1.5
Hulsea nana																0.2		
Ipomopsis congesta									1.6	0.8				2.4	3.2		0.4	

TABLE 5. Continued.

Botrychium pumicola (P/A)	P	A	P	A	P	A	P	A	P	A	P	A	P	P	A	P	A	P
Plant Associate	LW	LW	LE	LE	BR	BR	DO	DO	GR	GR	DU	DU	CN	SK	SK	BA	BA	SH
Linanthus pungens														3.8	5.8			
Lomatium martindalei	1		0.2															
Lupinus lepidus	1.3	1.9	4.9	9.5	1.4	0.2	0.6	0.4	7.6	21	8	10	7.6	14.6	5.8			
Machaeranthera canescens var. *shastensis*		2	0.3	1.2					1.2	1.2	1							
Mimulus nanus														0.8	0.6			
Phacelia hastata		1.1	0.8	7.5			4	1.4	3.6					3.6	6.6			
Phlox diffusa	1.3		3.3	7.4														
Phlox hoodii	8.6	17.5					37.8	25.4	3.4	2.2			7.2					
Phoenicaulis cheiranthoides													0.6					
Potentilla sp.													8.4					
Penstemon davidsonii					8	3.6								0.2	0.2	15	30	3
Penstemon speciosus							0.6											
Pinus contorta							0.2		0.2					0.2				
Polygonum newberryi						0.2												
Raillardella argentea	94.5	26.8	113.5	43.8							15	20						
Ribes erythrocarpum									0.2									
Senecio canus					1.2		0.4		0.2				6		2.8	0.8	2.4	
Trisetum spicatum				0.1												1.4		

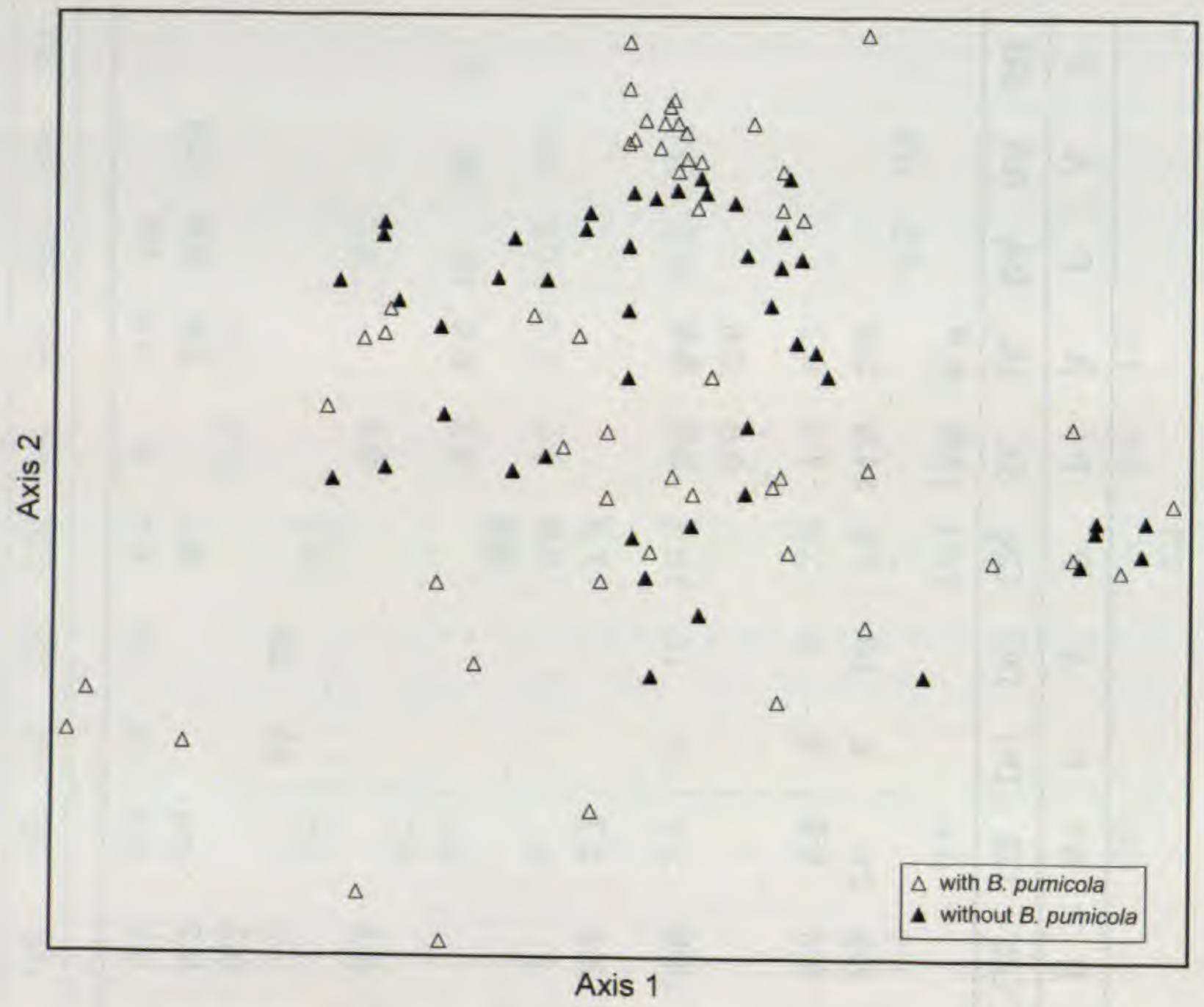

FIG. 4. NMS ordination for *Botrychium pumicola* populations in the Cascade Range of central and southern Oregon. All plant associates in greater than 5% of plots, five replicates per site; ten replicates for Llao Rock; Δ = with *B. pumicola*, ▲ = without *B. pumicola*.

TABLE 6. Nearest neighbor distances and indices for alpine and subalpine populations of *Botrychium pumicola* in the Cascade Range of central and southern Oregon and northern California. Average nearest neighbor distance (mean and sd) and index (NNI) between adjacent plants of *B. pumicola* and between *B. pumicola* and the nearest other species. Dutton Ridge (DU) dispersion could not be calculated due to a sample size of one. Values near zero indicate clumped distribution, values near one indicate random distribution, and values near 2.15 indicate uniform distribution.

	Nearest neighbor distance (cm), mean (sd)		Nearest neighbor index	
Population	Between adjacent *B. pumicola* plants	Between *B. pumicola* and other species	Between adjacent *B. pumicola* plants	Between *B. pumicola* and other species
DU	n/a	5.0	n/a	0.74
SH	9.1 (5.8)	1.6 (0.1)	0.34	0.10
BR	12.7 (7.5)	15.4 (8.2)	0.61	2.04
DO	13.3 (4.7)	5.5 (2.7)	0.68	0.95
BA	16.4 (6.5)	6.7 (5.7)	0.74	0.73
LW	17.5 (4.9)	2.5 (1.2)	1.10	0.69
GR	71.6 (37.3)	5.0 (2.6)	1.15	0.68
CN	22.9 (2.3)	4.4 (1.3)	1.17	0.88
LE	25.1 (13.8)	2.3 (0.8)	1.22	0.66
SK	44.6 (38.5)	4.6 (1.8)	1.33	0.86

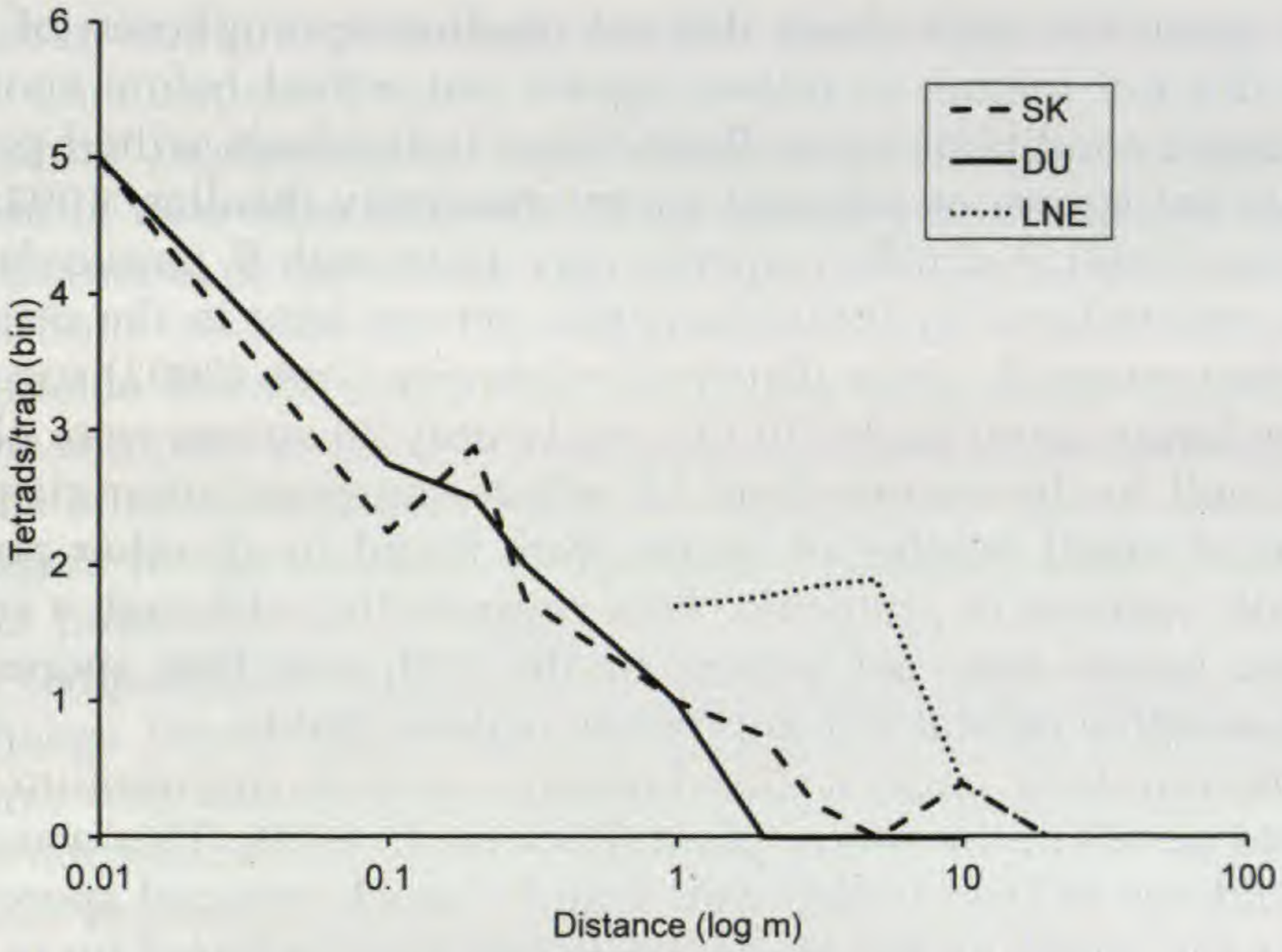

FIG. 5. Dispersal distances of *Botrychium pumicola* spore tetrads in Crater Lake National Park, Oregon. Dutton Ridge (DU); Skell Head (SK); Llao Rock Northeast (LNE).

Microscale slope and aspect may influence soil moisture and soil temperature creating patches of suitable habitat. *Botrychium pumicola* populations occurred on slopes of 0–27°. Populations of *B. pumicola* occurred on all aspects except northwest, with south, southwest, and west aspects more common. Snow distribution, snow pack duration and relief-driven summer precipitation strongly influence moisture availability during the growing season (Körner, 1999). Soil temperatures were lower in plots with *B. pumicola*.

Botrychium pumicola grows with diverse herbaceous perennial plants typical of south-central Cascadian high-elevation pumice fields and volcanic rubble, commonly *Carex breweri*, *Elymus elymoides*, *Eriogonum umbellatum* and *Lupinus lepidus* (Roe-Andersen, 2010). No plant species consistently occurred with *B. pumicola*. The difference in plant community composition in plots with and without *B. pumicola* was driven by singletons, which when removed showed no difference between plots. The high density of *Raillardella argentea* at Llao Rock with the largest *B. pumicola* population (n >1300) suggests that it may be an important mycorrhizal network partner.

Nearest neighbor analysis of *B. pumicola* and its nearest plant associate showed that in all populations, except at Broken Top, the plants had clumped dispersion. A nearest neighbor analysis of *B. pumicola* with conspecifics showed clumped and random dispersions. Conspecific clumped distribution suggests that gemmae and spores falling close to the parent plant dominate reproduction and that longer distance dispersal and establishment are less important. Clumping could also facilitate mycorrhizal inoculation of spores (Johnson-Groh and Lee, 2002). Random distribution indicates that *B. pumicola* is not required to be near a conspecific (or other AM host) to establish successfully.

Some *B. pumicola* individuals did not produce sporophores; of those that did, some did not mature to release spores but wilted before spore release. During drought conditions some *Botrychium* individuals wilted prematurely and did not set spores or released spores passively (Muller, 1992; Johnson-Groh and Lee, 2002). A similar response may occur with *B. pumicola* in the dry conditions encountered by individuals that emerge later in the season.

Botrychium pumicola spore dispersal.—Johnson-Groh (2002) suggested that *Botrychium* forms spore banks. In this study only 20 spores were observed in 250 mL of soil in the sample from LE where the population size is >1000 individuals. A small number of spores were found in all other populations, even in plots without *B. pumicola*. This suggests that although a small spore bank forms, spores may not persist in the soil, and that spores reaching apparently suitable habitat fail to produce mature plants.

In a spore trap study under a closed canopy, most *B. virginianum* spores fell within three meters of the source plant (Peck *et al.*, 1990). This was consistent with the findings of Dyer (1994) who found a much reduced spore bank two meters from the source. In this study, spore tetrads were found up to 10 m from a source plant, with most tetrads lodged within 30–100 cm. This supports a leptokurtic pattern of *Botrychium* spore dispersal with most spores lodging close to source plants (Peck *et al.*, 1990).

Rarity.—According to Rabinowitz's (1981) classification of species abundance, *B. pumicola* falls into the category of a species with both a narrow geographic range and narrow habitat specificity. Rarity of a species may be influenced by the size and number of habitable sites, by the carrying capacity of the sites, and by the length of habitability and the probability of dispersal to those habitats (Harper, 1981). *Botrychium pumicola* appears limited to volcanic substrates with appropriate soil nutrients, temperature, and density, and may also be limited by spore dispersal into suitable habitat. Due to the windy nature of exposed volcanic substrates, spores may periodically land in suitable habitat making the potential for colonizing distant habitats seem high. However, the fragmented distribution of populations suggests that, although dispersal occurs, the species encounters limitations in its ability to establish. High mortality from gametophyte to emergent sporophyte has been observed in other *Botrychium* species (Johnson-Groh *et al.*, 2002). If *B. pumicola* spores reach suitable habitat, the potential for failure of spores, gametophytes and juvenile sporophytes may be high.

Botrychium pumicola may be a "neo-endemic," a relatively new species that has not had time to expand its range (Fiedler, 2001). It may also be recovering from bottleneck effects due to past volcanic eruptions. The rediscovery of *B. pumicola* on Shastina Cone (Farrar, 2011; Farrar, 2012) adds a fascinating layer to the ecology of *B. pumicola* and opens a new chapter in the evolution and distribution of the species thought to be an Oregon endemic. Although *B. pumicola* is restricted to select volcanic habitats in the Cascade Range of central and southern Oregon and extreme northern California, the Shastina Cone population confirms that the species is not restricted to pumice substrates as previously reported (Wagner, 1990; Wagner and Wagner, 1993). Because the Shastina Cone plants have the same single genotype as the Oregon

plants (Farrar, 2011), a rare long distance spore dispersal event may have supported colonization of newer volcanic substrates in southern Oregon.

Conservation implications.—Long distance spore dispersal may allow for colonization of new sites, however, *B. pumicola* spore dispersal is primarily local. Furthermore, *B. pumicola* may have specific microsite habitat requirements that limit colonization. The fragmented distribution of the species and limited suitable habitat may preclude *B. pumicola* from establishing new populations at a pace to match the rate of floristic shifts accompanying climate change. The effects of climate change on *Botrychium* species are a concern of land managers in the western United States (Anderson and Cariveau, 2004). *Botrychium pumicola* may be negatively affected by increased CO_2, increased maximum temperatures and decreased snow water equivalent expected with climate change (Mote, 2003; Mote *et al.*, 2005). Predicted higher maximum temperatures with concomitant decrease in summer moisture levels may push the species northward in latitude and up in elevation. Alternatively, a downward shift in plant species distribution may occur with changes in water availability (Crimmins *et al.*, 2011). Consequences of either scenario are a reduction in suitable habitat islands for colonization and increased risk of local extinction.

Conclusions.—Our results suggest that microsite factors (soil nutrients, soil density, nearest neighbor dispersion) and dispersal limitations are likely key factors influencing the distribution of *Botrychium pumicola*. The fragmented distribution of *B. pumicola* populations results from a convergence of abiotic and biotic factors that create habitat islands appropriate for the growth and development of *B. pumicola* gametophytes, sporophytes and their AM fungal symbionts. Narrow spore germination requirements combined with dispersal limitations may influence population expansion of obligate mycorrhizal ferns such as *B. pumicola*.

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Pellaea flavescens, a Brazilian Endemic, is a Synonym of Old World *Pellaea viridis*

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ABSTRACT.—A phylogenetic analysis of plastid *rbcL* sequences indicates that *Pellaea flavescens* from Brazil is nested within the Old World species *Pellaea viridis*. The morphology of these two species is similar and *P. flavescens* is here considered to be a synonym of the older *P. viridis*. This fern may have been introduced to and subsequently naturalized in Brazil or, alternatively, the natural result of long distance dispersal.

KEY WORDS.—Pteridaceae, cheilanthoid, *rbcL*, phylogeny, ferns

Pellaea flavescens Fée, described from Rio de Janeiro and recently found in São Paulo, Brazil (Prado and Hirai, 2011), is morphologically similar to *Pellaea viridis* (Forssk.) Prantl from Africa, Madagascar, the Comoros, and the Mascarenes (Moran and Smith, 2001). Nonetheless, Tryon and Tryon (1982) placed *P. flavescens* in *Pellaea* section *Ormopteris*, a small group of species mostly endemic to central Brazil, while referring *P. viridis* to *Cheilanthes*. Several recent phylogenetic analyses (Gastony and Rollo, 1995; Kirkpatrick, 2007; Prado *et al.*, 2007; Schuettpelz *et al.*, 2007; Eiserhardt *et al.*, 2011) have demonstrated that both *Pellaea* and *Cheilanthes*, as defined by Tryon and Tryon (1982), are polyphyletic. These studies also showed that *P. viridis* and *Pellaea* section *Ormopteris* are not especially close relatives. Previously sampled members of *Pellaea* section *Ormopteris* nested, albeit with poor support, within a large *Doryopteris* clade, sister to *Doryopteris* section *Lytoneuron* (Prado *et al.*, 2007). *Pellaea viridis* was resolved (support values were not reported) in a separate clade of primarily African species variously assigned to *Cheilanthes* and *Pellaea* (Eiserhardt *et al.*, 2011).

The morphological similarity of *Pellaea flavescens* and *P. viridis* is at odds with their presumed placement in separate, distantly related clades. However, *P. flavescens* itself has not been sampled in any previously published molecular phylogenetic study, leaving the question of its evolutionary position unanswered. Here, we examine the relationships of this species, and the taxonomic implications thereof, through an analysis of plastid *rbcL* sequences.

Materials and Methods

A plastid *rbcL* sequence was obtained from a recent collection of *Pellaea flavescens* following established protocols (Schuettpelz and Pryer, 2007). A dataset was then assembled, consisting of this sequence, 22 exemplars from clade D of Eiserhardt *et al.* (2011) obtained from GenBank, and an additional published sequence of *P. viridis* (Table 1). These sequences were manually aligned using Mesquite version 2.75 (Maddison and Maddison, 2011) and subsequently analyzed using MRBAYES version 3.2.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Regions at the ends of the alignment containing copious amounts of missing data were excluded, leaving a dataset of 1143 characters to which a GTR+G model of sequence evolution was assigned. Our Bayesian analysis comprised two independent runs, each utilizing four chains of two million generations. Trees were sampled every 1000 generations. To identify when the runs had reached stationarity, the standard deviation of the split frequencies between the two runs was examined, and the output parameter estimates were plotted using Tracer 1.5 (Rambaut and Drummond, 2009). Based on these convergence diagnostics, the first 500 trees were (very conservatively) excluded from each analysis before obtaining a majority rule consensus phylogeny with clade posterior probabilities. The resulting tree was rooted with *Trachypteris pinnata* (Hook. f.) C. Chr.

Results and Discussion

The *rbcL* sequence obtained from our Brazilian *Pellaea flavescens* sample was identical to two Old World exemplars of *P. viridis* (EF452147 and GU935494; Table 1). These sequences compose a well-supported (posterior probability, PP = 1.00) clade in our recovered phylogeny (Fig. 1) that is, in turn, robustly supported (PP = 1.00) as sister to a third exemplar of *P. viridis*. *Pellaea* section *Ormopteris*, here represented by *P. gleichenioides* (Gardn.) Christ and *P. pinnata* (Kaulf.) Prantl, is not closely related to this clade. Instead, it is well-supported as sister to *Doryopteris* section *Lytoneuron* (PP = 1.00), consistent with the findings of Prado *et al.* (2007). Thus, the placement of *P. flavescens* in *Pellaea* section *Ormopteris* by Tryon and Tryon (1982) is not supported by our molecular analysis.

The phylogenetic position of *Pellaea flavescens* as closely related to *P. viridis*, is supported by morphology. In fact, these taxa were recognized as a "species pair" by Moran and Smith (2001), who noted only slight structural differences. Here, however, we find *P. flavescens* to be nested within *P. viridis*, having an *rbcL* sequence identical to those of two *P. viridis* individuals (Fig. 1). Although *rbcL* is among the more slowly evolving plastid genes, a recent study of candidate barcodes (Li *et al.*, 2011) found it to be comparable to a much more rapidly evolving gene (*matK*) in its discriminatory power. Upwards of 40% of the species in the *Cheilanthes marginata* group (now recognized as the genus *Gaga*; Li *et al.*, 2012) could be separated based on *rbcL* alone (Li *et al.*, 2011). Unfortunately, but not surprisingly, discriminatory power was weakest

TABLE 1. List of sampled taxa, including GenBank accession numbers, source localities, and voucher information.

Species	Accession	Locality	Voucher information
Adiantopsis chlorophylla (Sm.) Fée	EF473684	Brazil	Prado *et al.* (2007)
Adiantopsis radiata (L.) Fée	EF452131	Guadeloupe	Schuettpelz *et al.* (2007)
Cheilanthes flexuosa Kunze	EF473686	Brazil	Prado *et al.* (2007)
Cheilanthes goyazensis (Taub.) Domin	EF473687	Brazil	Prado *et al.* (2007)
Cheilanthes induta Kunze	GU935501	South Africa	Eiserhardt *et al.* (2011)
Cheilanthes multifida (Sw.) Sw.	GU935499	South Africa	Eiserhardt *et al.* (2011)
Cheilanthes namaquensis (Baker) Schelpe & N.C. Anthony	GU935492	South Africa	Eiserhardt *et al.* (2011)
Cheilanthes quadripinnata (Forssk.) Kuhn	GU935496	South Africa	Eiserhardt *et al.* (2011)
Cheilanthes venusta Fée	JN122014	Brazil	Eiserhardt *et al.* (2011)
Doryopteris collina (Raddi) J. Sm.	EF473688	Brazil	Prado *et al.* (2007)
Doryopteris concolor (Langsd. & Fisch.) Kuhn	U05621	Taiwan	Hasebe *et al.* (1994)
Doryopteris lomariacea Klotzsch	EF473689	Brazil	Prado *et al.* (2007)
Doryopteris ornithopus (Hook. & Baker) J. Sm.	EF473691	Brazil	Prado *et al.* (2007)
Doryopteris pedata (L.) Fée	U27206	Mexico	Gastony and Rollo (1995)
Doryopteris pentagona Pic. Serm.	EF473693	Brazil	Prado *et al.* (2007)
Pellaea calomelanos (Sw.) Link	GU935497	Namibia	Eiserhardt *et al.* (2011)
Pellaea flavescens Fée	JX455163	Brazil	Prado 2036 (SP)
Pellaea gleichenioides (Gardn.) Christ	EF473698	Brazil	Prado *et al.* (2007)
Pellaea pinnata (Kaulf.) Prantl	EF473699	Brazil	Prado *et al.* (2007)
Pellaea pteroides (L.) Prantl	GU935502	South Africa	Eiserhardt *et al.* (2011)
Pellaea viridis (Forssk.) Prantl	EF452147	Réunion	Schuettpelz *et al.* (2007)
Pellaea viridis (Forssk.) Prantl	GU935494	South Africa	Eiserhardt *et al.* (2011)
Pellaea viridis (Forssk.) Prantl	GU935495	South Africa	Eiserhardt *et al.* (2011)
Trachypteris pinnata (Hook. f.) C. Chr.	U27450	Bolivia	Gastony and Rollo (1995)

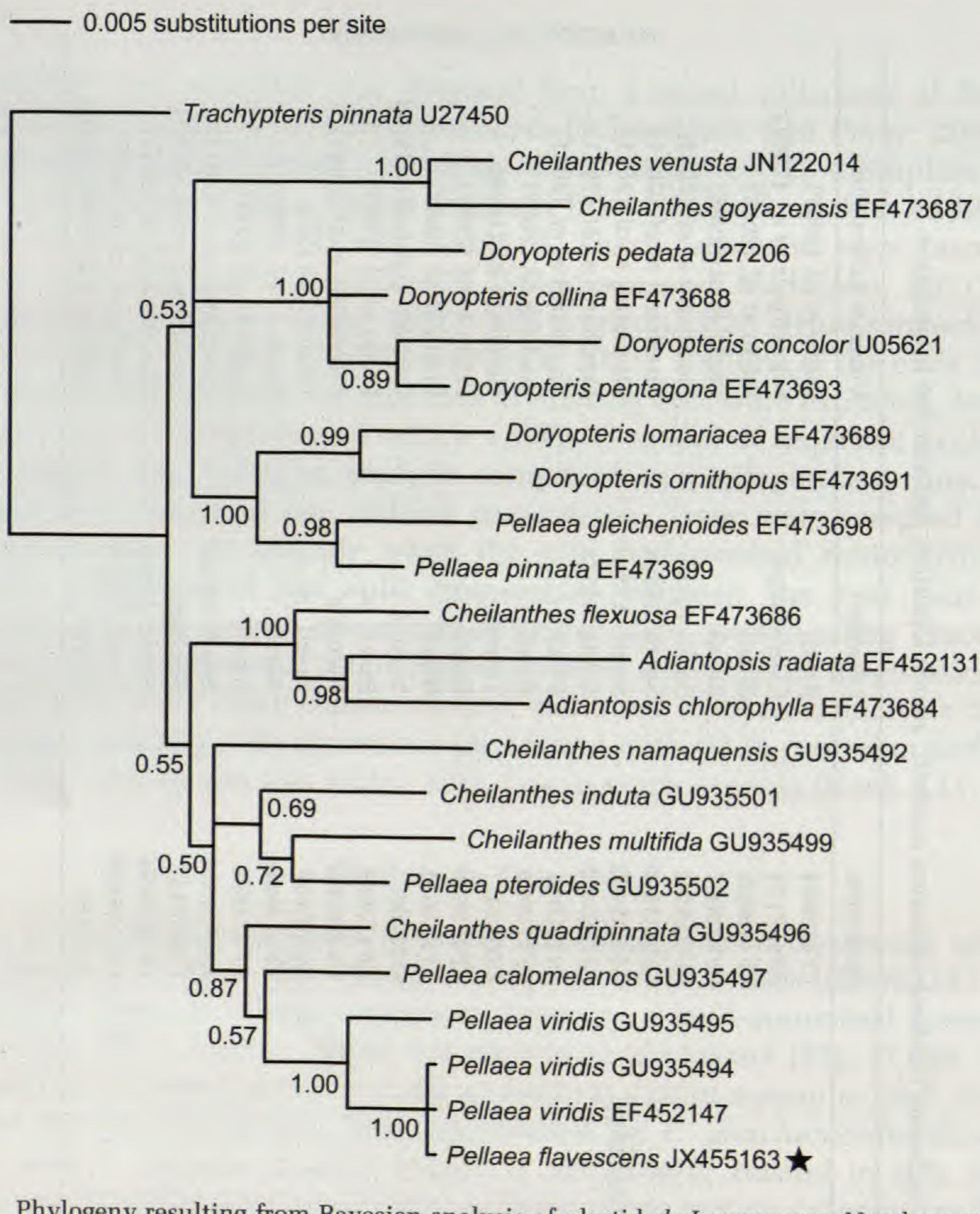

FIG. 1. Phylogeny resulting from Bayesian analysis of plastid *rbcL* sequences. Numbers at nodes are posterior probabilities. Brazilian *Pellaea flavescens*, here recognized as a synonym of Old World *P. viridis*, is marked with a star.

within species complexes. Nonetheless, based on the available evidence, and pending more exhaustive sequencing and a detailed study of the *Pellaea viridis* complex (see below), we recommend treating *P. flavescens* and *P. viridis* as synonyms, with the latter having priority.

The members of what is herein referred to as the *Pellaea viridis* complex will eventually need to be accommodated in some genus other than *Pellaea* or *Cheilanthes*, as the types of these genera are resolved elsewhere in the cheilanthoid phylogeny (Eiserhardt *et al.*, 2011; Kirkpatrick, 2007). Another point of nomenclatural clarification concerns *P. bongardiana* Baker, the description of which cited the type of *P. flavescens*. The former name is

therefore superfluous and cannot be used unless conserved or sanctioned, according to the International Code of Nomenclature for algae, fungi, and plants (McNeill *et al.*, 2012). In any case, the figure cited for *P. bongardiana* in the *Flora Brasiliensis* (tab. 55, fig. II) is wrong, with the illustrated plant representing *Pellaea riedelii* Baker.

Pellaea viridis is commonly cultivated (Hoshizaki and Moran, 2001), and escape from cultivation, followed by naturalization, has been documented (e.g., in Hawaii, by Palmer, 2003; in eastern Australia, by Bostock, 1998; and in Florida, see Atlas of Florida vascular plants: http://florida.plantatlas.usf.edu/). Thus, it may be that the existence of this species in Brazil represents yet another introduction from cultivation into the wild. Despite this apparently reasonable possibility, *P. viridis* is not currently cultivated in Brazil, so far as we are aware, and the Brazilian populations generally occur in undisturbed environments. Furthermore, while the first Brazilian collection (*Glaziou 2473*) we have seen dates from 1867, the current distribution of the species in Brazil, nearly 150 years later, remains highly restricted (Prado and Hirai, 2011). This stands in stark contrast to what has been encountered for most other introduced species. For example, despite a much more recent introduction into the New World of the Old World fern *Thelypteris dentata* (Forssk.) E. P. St. John (the earliest New World collection dates from 1908; Strother and Smith, 1970), this species is today one of the most common ferns in many areas of the Neotropics (Mickel and Smith, 2004). Although the distribution of the apparently introduced *Marsilea hirsuta* in the Azores is even more restricted than that of *P. flavescens* in Brazil (Schaefer *et al.*, 2011), the absence of *Marsilea* in historical collections from the Azores would point to a very recent introduction of the genus there. It is possible, then, that the populations of *P. viridis* in Brazil are native, originating via long distance spore dispersal from the Old World. Although uncommon, such a disjunction is not unheard of: Moran and Smith (2001) documented 27 pteridophyte species that naturally occur in both the Neotropics and Africa.

Moving forward, it is clear that the *Pellaea viridis* complex is in need of further examination (Moran and Smith, 2001; Eiserhardt *et al.*, 2011), not only to clarify species boundaries but also to identify the affinities of the Brazilian and other New World collections. Only through a more thorough analysis will it be possible to uncover the true origin of this lineage in Brazil.

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Mating System in *Blechnum spicant* and *Dryopteris affinis* ssp. *affinis* Correlates with Genetic Variability

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Abstract.—The monilophytes *Blechnum spicant* (L.) Sm. and *Dryopteris affinis* ssp. *affinis* (Lowe) Fraser-Jenkins show different reproductive strategies under *in vitro* conditions. While *B. spicant* exhibits asexual and sexual reproduction, with an antheridiogen system promoting outcrossing, *D. affinis* ssp. *affinis* reproduces only asexually through apogamy. Individuals of several populations of these species, collected in Principado de Asturias (Spain), were analyzed to test the influence of their mating system in the genetic variability displayed by each species. This study shows that the genetic diversity assessed in populations of each species collected *in situ* is in concordance with that expected according to their reproductive system previously observed *in vitro*. The genetic diversity found in *D. affinis* ssp. *affinis* was low and most of genetic variation can be attributed to differences among localities. This result indicates high fixation of the detected alleles within each locality, as expected for a clonal reproductive system. In the sexual species *B. spicant* the genetic diversity was higher. Our results confirm the importance of reproduction system in the genetic diversity present in populations of these fern species making essential to consider the definition and study of reproductive system and the associated consequences in the design of successful conservation actions.

Key Words.—AFLP, *Blechnun spicant*, clonal growth, *Dryopteris affinis*, sexual reproduction

The formation of a new fern sporophyte can be achieved through asexual or sexual pathways. During asexual reproduction, cells other than gametes develop a sporophyte through an asexual process without meiosis or fertilization called apogamy. The fern complex *Dryopteris affinis* (Lowe) Fraser-Jenkins includes diploid and triploid subspecies, all of them apogamic (Fraser-Jenkins, 1980). Apogamy has been reported to be a way of escaping hybrid sterility by alloploids (Fraser-Jenkins, 1980; Chao *et al.*, 2012). On the other hand, the mating system of the fern species *Blechnum spicant* (L.) Sm. implies the development of gametophytes that produce male and female gametes in the sexual organs, archegonia and antheridia, which form a sexual embryo through fertilization.

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Gametophytes are relatively easy to growth under *in vitro* controlled conditions (Fernández and Revilla, 2003) allowing a detailed study of their developmental process. Apogamous sporophyte formation during *in vitro* cultivation of *Dryopteris affinis* ssp. *affinis* has been described (Fernandez *et al.*, 1996; Menendez *et al.*, 2006). Gametophyte development has also been described under *in vitro* conditions for *Blechnum spicant* (Fernandez *et al.*, 1997).

Although self-fertilization is common in many fern species, both inbreeding and outcrossing schemes are possible. To promote outcrossing, some homosporous fern species present morphological barriers to prevent self-fertilization, such as asynchronous maturation time of gametophytes. Maturing female gametophytes produce antheridiogens that induce the younger, adjacent gametophytes to develop into male gametophytes both *in vivo* and *in vitro* culture conditions (Klekowski, 1969; Cousens, 1979; Fernández *et al.*, 1997; Menéndez *et al.*, 2006) promoting by this way genetic exchange. Ranker and Houston (2002) enquired whether gametophyte sexuality in the laboratory would be a good predictor of the mating system in nature, finding that *in vitro* culture gametophyte studies would lead to the same main conclusions than those performed on field collected ones. Although some species present mixed mating systems, mating strategies in fern species vary from mainly self-fertilization to obligate intergametophytic crossing, in a bimodal fashion (Soltis and Soltis, 1990, 1992). Recent results show that mixed mating systems in the same species are more frequent than previously reported, with high selfing capacity as a strategy for long dispersal and colonization (de Groot *et al.*, 2012).

The breeding system has been reported as an important determinant of genetic variation in plants (Hamrick and Godt, 1996). Outcrossers usually present higher polymorphic loci or number of genotypes per population than inbreeders (Holsinger, 2000). High genetic diversity has been observed in homosporous pteridophytes with high degree of outcrossing (Maki and Asada, 1998; Kang *et al.*, 2005). Genetic uniformity within populations was found in ferns whose gametophytes reproduce through intragametophytic selfing as *Asplenium csikii* (Vogel *et al.*, 1999). Also intragametophytic selfing has been reported as a trait that could be linked to invasiveness and successful colonization of new habitats (Lott *et al.*, 2003). Asexual reproduction, on the other hand, has been associated with low genetic variability by decreasing the effective population size in species such as *Ceratopteris pteridoides* (Chen *et al.*, 2010).

In this study, the genetic variability present in natural populations of two ferns representing asexual and sexual mating systems, *D. affinis* ssp. *affinis* and *B. spicant,* was assessed using AFLPs to test the repercussions of the reproductive biology on their genetic variability.

Materials and Methods

Sample collection and DNA extraction.—Two fern species characterized by different reproductive systems were selected for the present study, *Blechnum spicant* (L.) Sm. and *Dryopteris affinis* ssp. *affinis* (Lowe) Fraser-Jenkins. For

each species, plants growing in three separated localities were analyzed (Table 1). To avoid problems of repetition of samples due to the possible re-sampling of the same individual (i.e. rhizome mediated asexual reproduction), a safety distance was kept between each selected individual for sampling (Table 2). The number of samples used in the genetic fingerprinting analysis varied from four to ten plants per population (Table 1). Samples were individually stored at −80°C until DNA extraction.

Molecular analysis.—Total genomic DNA of each individual plant was extracted using a DNeasy Plant mini kit (Qiagen Ltd, Crawley, UK). AFLP analysis was performed according to Peredo *et al.* (2008). For each sample, AFLP analysis was performed using MseI and EcoRI digests. The pre-selective and selective amplifications were conducted using standard AFLP cycling parameters including a touch-down selective PCR. The primers used for the pre-selective amplification were EcoRI + A, and MseI + C, while for the selective amplification the primers used were *Eco* AGC/*Mse* CAT, AGC/CTT, AGA/CAT, AGA/CTT, AAC/CTT, ACC/CAT, and ACC/CTT. After PCR, samples were denatured by adding an equal volume of formamide buffer (98% formamide, 10 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol, pH 8.0) and heated for 3 min at 94 °C. Aliquots (6 ul) of each sample were loaded on 6% polyacrylamide gels (acrylamide/bisacrylamide; 19:1) containing 7.5 M urea and 1 x TBE, and then electrophoresed for 2 h. A standard silver staining protocol was used to reveal the bands: 20 min in fixing solution 1 (acetic acid 1% ethanol 10%), 3 min in fixing solution 2 (nitric acid 1.5%), 20 min in impregnate solution (silver nitrate 2 g/L), and developed in sodium carbonate (30 g/L) supplemented with 0.27 ml/L formaldehyde. The gel was rinsed in deionized water after each step.

Data analysis.—The presence/absence of AFLP bands was determined by visual inspection and scored as 1/0. Only the bands showing a clear and easily detectable signal were taken into account.

A first evaluation of the data was performed by scoring the number of detected loci per primer combination in each population and species. The percentage of monomorphic loci was evaluated for primer combination and population in all the analyzed samples with or without including singletons. A singleton is the presence of a locus alteration in a single individual, that is, the presence or absence of a band. The correction was applied to minimize the possible error due to scoring or other technique related mistakes. Additional precautions were taken to minimize the technical error such as a duplicate DNA extraction for each individual, which were treated as independent samples through all the molecular procedures to assess data repetitivity. All the data used in this study present less than one discrepancy per each 50 loci (over 95% repetitivity).

Estimates of genetic diversity within populations, including percentage of polymorphic loci, Shannon's index (Shannon and Weaver, 1949), and Nei's gene diversity assuming random mating (Nei, 1973), were calculated using the software POPGENE version 1.31 (Yeh and Boyle, 1997).

TABLE 1. List of sampled sites for *Blechnum spicant* (B) and *Dryopteris affinis ssp.affinis* (D) populations.

Species	Population	Sampling site	UTM coordinates (30T)	Lat/Lon Coordinates	Individuals (n)	Habitat	Date
Blechnum spicant	B 1	Nueva, river Nueva	0342868 4810718	43° 25′ 58.327″ N 4° 56′ 29.279″ W	10	Alder forest (*Alnus glutinosa*)	15/1/2009
	B 2	Novales river	0366566 4805620	43° 23′ 29.706″ N 4° 38′ 51.171″ W	10	Alder forest	
	B 3	Road to Purón	0362878 4806340	43° 23′ 50.639″ N 4° 41′ 35.684″ W	10	Shadow slope, heath	
Dryopteris affinis* ssp. *affinis	D 8	Nueva, river Nueva	0342852 4810789	43° 26′ 0.615″ N 4° 56′ 30.064″ W	4	Alder foresta	12/3/2009
	D 9	Gobiendes	0319177 4814827	43° 27′ 52.154″ N 5° 14′ 7.228″ W	4	Alder forest	
	D 10	Purón river, road to Purón	0362944 4805766	43° 23′ 32.083″ N 4° 41′ 32.233″ W	5	Alder forest	

TABLE 2. Distances (km) among different sampling sites of *Blechnum spicant* (B) and *Dryopteris affinis ssp.affinis* (D).

B	B1	B2	B3	D	D8	D9	D10
B1	-			**D8**	-		
B2	24.21	-		**D9**	24.48	-	
B3	20.43	3.8	-	**D10**	20.54	45.33	-

Data were analyzed with the program STRUCTURE (Pritchard et al, 2000), which employs a model-based Bayesian clustering procedure to determine the number of divergent groups (K) in a dataset. Ten independent runs were undertaken with K varying from 1 to 5. Each run was performed with 30,000 MCMC (Markov Chain Monte Carlo) repetitions following a burn-in of 30,000 iterations. The analysis used no prior information, assumed correlated allele frequencies and both admixture and no admixture. Posterior probabilities were calculated for each value of K, and the estimated ΔK (Evanno *et al.*, 2005) was used to choose the optimal K value. Following the analysis by STRUCTURE, data were also subjected to a standard analysis of molecular variance (AMOVA) using ARLEQUIN version 3.2 (Excoffier *et al.*, 2005) to assess the level of differentiation within and among different groupings of populations defined "a posteriori". A principal coordinate analysis (PCoA) was conducted on a similarity matrix using the Dice's index [$djk = 2M / (2M+N)$. where M is the number of matches and N is the total number of columns with presence in just one row] constructed from the primary binary dataset using the program PAST (version 1.54) (Hammer *et al.*, 2006). In addition, cluster analysis was performed on a matrix of distance values between individuals using paired group algorithm for binary data (presence/absence) with Dice distance as implemented in the PAST program (bootstrapped 1,000 times). To confirm the reliability of the groupings, trees were calculated for each primer combination and for the total detected loci.

RESULTS

Estimates of genetic diversity.—In the case of *Blechnum spicant*, 11 primer combinations of those tested were selected for the study based on their repetitivity. A total of 623 loci was detected (Table 3), and the number of loci per primer combination ranged from 35 (EAGAMCAT) to 85 (EACCMCTT) with a mean of 56.5±15.7. The mean percentage of monomorphic loci detected per primer combination in all the populations was 17.6% (ranging from 4.7% to 36.6%). To minimize the effect of possible artifacts that might lead to an overestimation of the polymorphisms, an additional calculation was done; the correction consisted in not considering singletons. The mean percentage of monomorphic sites increased to 26.32% (6.1–45.1%). The percentages were also calculated for each population: B1 39.17% (25.9–60.8%); B2 54.25% (26.4–79.2%) and B3 42.54% (11.6–69%).

TABLE 3. Genetic indexes, Nei's (HE) and Shannon's (I), and percentage of polymorphism (%P) calculated from AFLP analysis for *Blechnum spicant* (B1, B2, B3, total in grey) and *Dryopteris affinis* ssp *affinis* (D8, D9, D10, total in grey) using POPGENE version 1.31 (Yeh and Boyle 1997).

AFLP primers (EcoRI/ MseI)	*Blechnum spicant*											
		HE				*I*				*%P*		
	N loci	B1	B2	B3	B	B1	B2	B3	B	B1	B2	B3	B
AAC/CAT	72	0,18	0,10	0,10	0,19	0,28	0,16	0,16	0,30	54,17	29,17	30,56	63,89
AAC/CTT	53	0,19	0,07	0,13	0,25	0,28	0,11	0,20	0,37	49,06	20,75	37,74	69,81
AAC/TCC	72	0,19	0,23	0,22	0,27	0,30	0,36	0,34	0,42	59,72	73,61	70,83	94,44
ACC/CAT	42	0,23	0,21	0,21	0,25	0,36	0,33	0,33	0,40	73,81	71,43	71,43	88,10
ACC/CTT	85	0,24	0,21	0,21	0,26	0,37	0,32	0,33	0,40	72,94	65,88	70,59	91,76
ACG/CAT	51	0,13	0,08	0,14	0,16	0,20	0,12	0,22	0,27	41,18	27,45	50,98	72,55
ACT/CAT	43	0,17	0,09	0,27	0,21	0,27	0,13	0,40	0,35	58,14	27,91	76,74	88,37
AGA/CAT	35	0,16	0,13	0,14	0,19	0,24	0,18	0,21	0,29	48,57	28,57	37,14	60,00
AGA/CTT	63	0,18	0,09	0,21	0,24	0,29	0,14	0,31	0,37	58,73	30,16	57,14	84,13
AGA/TCC	65	0,23	0,24	0,22	0,34	0,35	0,36	0,33	0,51	67,69	72,31	67,69	95,38
AGG/CTT	42	0,22	0,08	0,13	0,18	0,34	0,13	0,20	0,29	69,05	28,57	40,48	71,43
TOTAL	623	0,20	0,15	0,18	0,24	0,30	0,22	0,28	0,37	60,03	45,75	56,50	81,38
	Dryopteris affinis ssp *affinis*											
	n loci	D8	D9	D10	D	D8	D9	D10	D	D8	D9	D10	D
AAG/CAT	107	0,15	0,05	0,01	0,15	0,22	0,07	0,01	0,25	34,58	11,21	1,87	56,07
AAG/CTT	85	0,31	0,02	0,01	0,20	0,44	0,03	0,01	0,32	68,24	4,71	2,35	74,12
AAC/CAT	70	0,05	0,11	0,05	0,21	0,07	0,15	0,08	0,31	11,43	24,29	12,86	57,14
AAC/CTT	44	0,05	0,17	0,06	0,16	0,08	0,25	0,09	0,25	13,64	38,64	15,91	54,55
AAC/TCC	50	0,00	0,15	0,05	0,22	0,00	0,22	0,07	0,33	0,00	34,00	12,00	66,00
ACC/CAT	62	0,06	0,01	0,04	0,14	0,08	0,01	0,06	0,21	12,90	1,61	11,29	38,71
ACC/CTT	63	0,01	0,00	0,00	0,05	0,01	0,00	0,00	0,07	1,59	0,00	0,00	12,70
ACC/TCC	33	0,03	0,00	0,01	0,11	0,04	0,00	0,02	0,16	6,06	0,00	3,03	27,27
AGA/CTT	71	0,21	0,03	0,00	0,20	0,30	0,04	0,01	0,32	46,48	7,04	1,41	71,83
AGC/TCC	71	0,21	0,01	0,03	0,18	0,30	0,02	0,04	0,29	49,30	2,82	7,04	66,20
TOTAL	656	0,13	0,05	0,02	0,16	0,18	0,07	0,04	0,26	28,66	11,43	6,10	54,73

For *Dryopteris affinis* ssp. *affinis*, 10 primers were selected using the same criterion detailed above. The total number of loci was 656, mean 65.6±20.97 ranging from 33 to 103. The mean percentage of monomophic loci detected in all the pooled populations was 46.4% (25.9 to 87.3%) and increased to 61.7% (42.1 to 88.9%) when singletons were not taken into account. The average percentages calculated for each *Dryopteris* population were: D8 75.3% (31.8–100%); D9 85.8% (61.4–100%), D10 92.5% (84.1–100%).

Two genetic indexes, Nei's (HE) and Shannon's (I), were calculated for each primer combination in each population and per species. Instead of calculating the average for the indexes, they were recalculated using total detected loci per primer combination and in total (Table 3). The total HE for *B. spicant* was 0.24 ranging from 0.16 (EACGMCAT) to 0.34 (EAGAMTCC), whereas in *D. affinis* ssp. *affinis* it was 0.16 (0.05–0.21). In the case of I, 0.37 (0.27–0.51) was

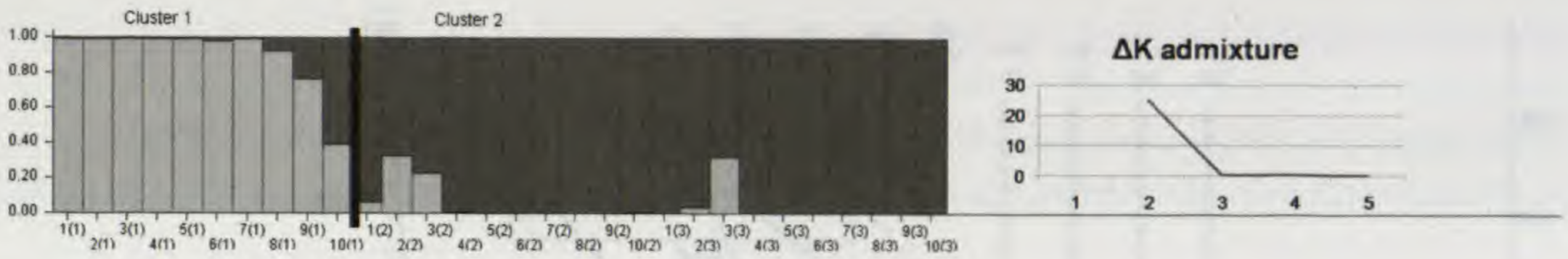

FIG. 1. Left: Bar plot of the proportion of an individual's of *Blechnum spicant* genome belonging to one or other cluster inferred by the STRUCTURE analysis. Cluster 1 is represented in green and includes individuals from B1 (Nueva) sampled site while cluster 2 is represented in red and includes individuals from sampled sites B2 (Novales) and B3 (Purón). Right: Plot of ΔK for each K value calculated as described in Evanno *et al.* (2005).

calculated for *Blechnum* and 0.26 (0.07–0.33) for *Dryopteris*. HE and I calculated for each population were: B1 0.20, 030; B2 0.15, 0.22; B3 0.18, 0.28; D8, 0.13, 0.18; D9 0.05, 0.07, and D10 0.02, 0.04 (consult Table 3 for a detailed list).

To test the existence of significant differences between the HE, I, and percentage of polymorphism (%P) values calculated for each species, the Mann-Whitney U test and Krustal-Wallis test were applied. Firstly, the suitability of the data to these tests was corroborated by checking whether the data fit to a normal distribution. To eliminate the possibility of variation due to other causes than the species as a source for the differences in the genetic variation (e.g. primer combinations chosen or population related variation) several tests were performed prior the comparison species to species. No significant differences were detected in any of the comparison within *D. affinis* ssp. *affinis* (data not shown), whereas for *B. spicant* statistically significant differences (%P $p = 0.03$) were found only in the percentage of polymorphism calculated for each primer combination. No significant differences were found using the same approach (Krustal-Wallis test) to the values calculated within each species.

The existence of differences between the values of heterozygosity (HE), Shannon index (I), and percentage of polymorphism (%P) in *D. affinis* ssp. a*ffinis* and *B. spicant* was tested using two-tailed Mann-Whitney U test. Significant differences were detected in each comparison: HE $p<0.0001$ (Monte Carlo $p<0.0001$); I $p<0.0001$ (Monte Carlo $p<0.0001$); and %P $p<0.0001$ (Monte Carlo $p<0.0001$).

Genetic structure in Blechnum spicant *and* Dryopteris affinis.—The Bayesian-clustering was applied to the datasets as implied in the STRUCTURE program assuming an admixture model, with the assumption of the possibility of mixed ancestry of the individuals. In *B. spicant*, Evanno's ΔK indicate the possibility of a $K=2$ grouping as the value for $\Delta K = 2$ was 25.4 and low values were calculated for $\Delta K > 2$ (<0.5) (Fig. 1). In each of the 10 replicates calculated for $K=2$ individuals from the locality B1 (Nueva) grouped in cluster 1 with a high probability (>0.904) while individuals from B2 (Novales) and B3 (Road to Puron) belonged to cluster 2 (probability over 0.928 and 0.958, respectively). This high degree of similarity among the individuals from populations B2 and B3 was also confirmed by the detected grouping in the

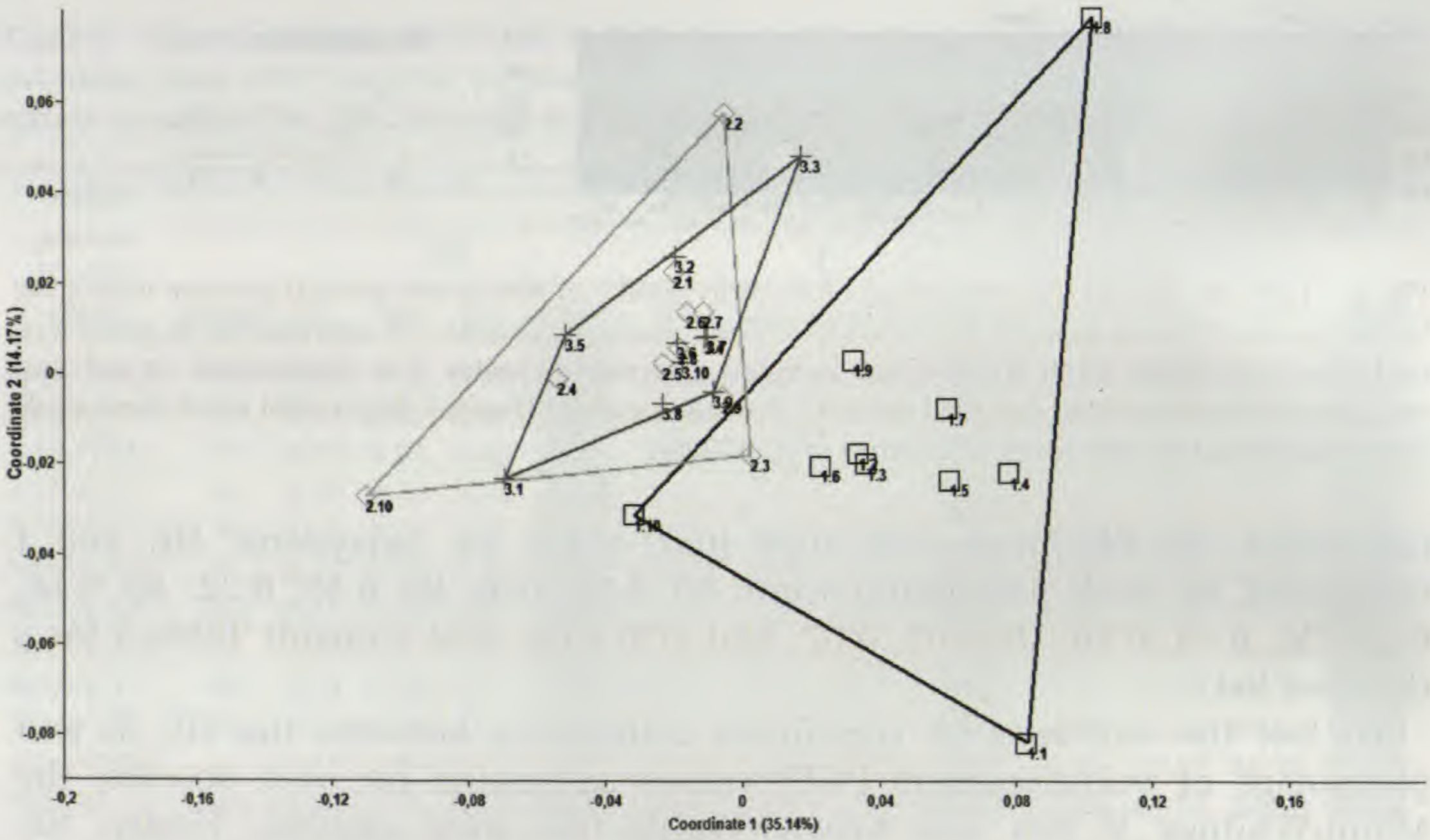

FIG. 2. Plot of principal coordinates 1 vs. 2 calculated using Dice's distance. Blue squares represent individuals from B1, green rhombus from B2, and red crosses from B3.

Principal Coordinates analysis (Fig. 2). Specific Fst values calculated for each sampled site were B1=0.26, B2=0.28 and B3=0.27. The *a posteriori* groupings of the sampling sites tested included an only group enclosing the three sampling sites, and two groups exploring the possible groupings (B1 vs B2 and B3; B1 and B2 vs B3; and B1 and B3 vs B2). No further statistical significance was found of population grouping detected by STRUCTURE (B1 vs B2 and B3) probably due to the amount of variation (over 60%) attributed to differences within populations.

For the *Dryopteris affinis* ssp. *affinis* populations, the Bayesian-clustering method employed in STRUCTURE failed to unequivocally demonstrate the existence of any clustering ($K > 1$) presents in the AFLP dataset probably due to the small sample size. While Evanno's ΔK might indicate the existence of two groups, as very low values were calculated for $K > 2$ (< 0.4), this result was not further confirmed by the probability of being assigned to each cluster calculated for each individual. While for individuals from populations D8 and D10 the probability of being assigned to cluster one or two was over 0.98, the individuals from D9 had similar probability of belonging to any of the clusters (aprox. 0.5).

This STRUCTURE result is in contradiction with the results by the AMOVA tests. The *a posteriori* groupings of the sampling sites tested included an only group enclosing the three sampling sites, and two groups (D8 vs D9 and D10; D8 and D10 vs D9; and D8 y D9 vs D10) indicated that there are differences among the sampling sites and there is no significant further grouping of the populations by geographical area indicating a high differentiation of each of them. Over a 60% of the variability is attributed to differences among

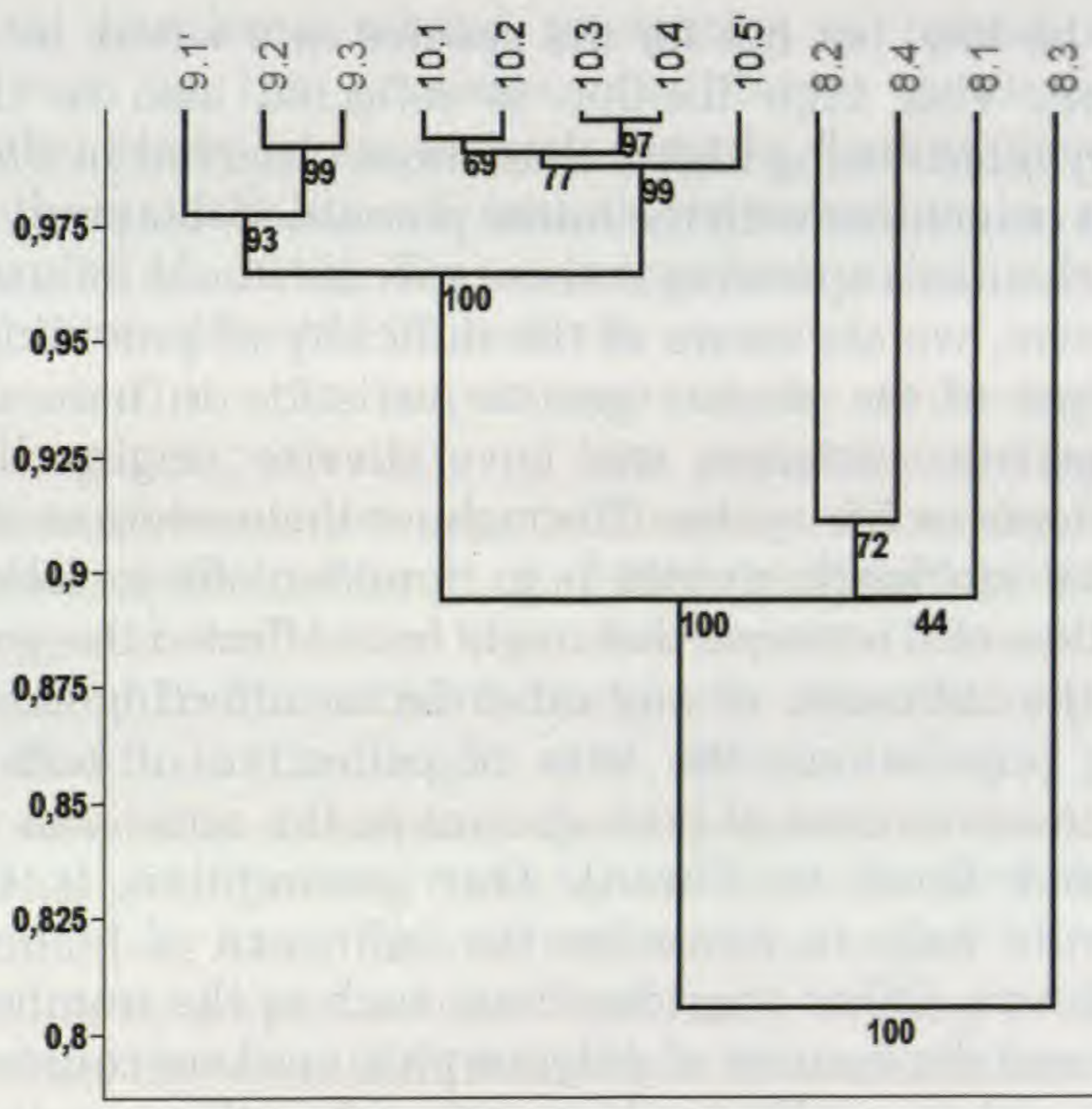

FIG. 3. Dendrogram calculated using Dice's distance as implied in PAST program representing the genetic relationships among *Dryopteris affinis* ssp. *affinis* individuals from the three sampling sites. Individuals from D8n are shown in green, D9 in pink, and D10 in blue. The bootstrap value is given in black in each node.

populations. The high Fst values also corroborate the high local fixation of variability (D8, 0.538; D9, 0.628; D10, 0.685). This high degree of similarity among the individuals from the same sampling site was confirmed by the high bootstrap support (>90%) of the different branches in the dendrogram calculated with PAST (Fig. 3) or the grouping in the PCoA (data not shown).

DISCUSSION

The genetic diversity calculated for the ferns *Blechnum spicant* and *Dryopteris affinis* ssp. *affinis* is in agreement with the reproductive mechanisms observed previously when cultured *in vitro* (Fernández and Revilla, 2003). Also, our results indicate the importance of small-scale sampling.

A relatively high percentage of polymorphism was estimated with AFLP for two species of ferns (81.38% for *B. spicant* and 54,73% for *D. affinis* ssp. *affinis*) in a small geographical area in the Principado de Asturias; no populations were found more than 45 km apart. However, when the distribution of this variation was analyzed in relation to the sampling sites, the polymorphism was drastically reduced to at least half and even one tenth in the case of *D. affinis* (28.66% (D8), 11.43% (D9) and 6.10% (D10). In the case of *B. spicant* a less notable effect was observed, with recalculated polymorphism ranging between 45.75% (B2) and 60.03% (B1). These changes in the percentage of polymorphism are explained by high genetic fixation, making the variation in each

sampling site to be low, but not for the species as a whole in this particular geographic region. This high fixation is reflected also in the Fst values calculated for *Dryopteris* being higher than those observed in *Blechnum*.

This fixation is coincident with the initial premise of this study, differences in reproduction mechanisms operating in these species would influence the genetic variability. However, we are aware of the difficulty of providing a conclusive probe of the origin of the present genetic variation in natural populations. Differences in genetic variation can have diverse origins beside specific reproduction systems or life cycles. Throughout the evolution of each species, it is possible that stochastic events (e.g., founder effect, selection of some genotypes or bottlenecks) occurred that might have affected the genetic diversity.

To minimize the influence of any other factor affecting the estimation of diversity among populations, the sites of collection of both species were selected by the co-occurrence of both species in the same area when possible (River Nueva and Road to Turon). Our assumption is that a shared environment would help to minimize the influence of biotic, abiotic, and anthropogenic factors. Other considerations such as the number of plants for each population and the number of polymorphic markers counted do not seem to produce any serious artifact when estimating the genetic intraspecific diversity in plants, as was shown by several studies using different dominant markers with this goal in gymnosperms and angiosperms (Nybom and Bartish, 2000). Finally, the variability could be caused by the experimental design (e.g. primer selection). Our analysis failed to detect any significant differences in the analyzed variables (HE, I and %P) that could be attributed to primer combinations used for each species or differences among populations within the same species. Our analysis, however, detected significant differences attributive to the two fern species in all estimates of genetic variability.

The different degree of genetic variation found in the natural populations of *B. spicant* and *D. affinis* ssp. *affinis* can be explained, then, to the differences in the reproductive biology of the species observed through *in vitro* culture. Traditionally, it has been maintained that most homosporous fern species are outbreeders or inbreeders (Soltis and Soltis, 1992) but more recent data suggest that the species could present a mix of reproductive strategies (de Groot *et al.*, 2012). Also, laboratory studies suggested that many fern species have mechanisms leading to the formation of functionally unisexual gametophytes that potentially could promote outcrossing. Because of the difficulty of working in *ex vitro* with gametophytes, not much work has been done to clarify whether outcrossing actually occurs in natural populations of ferns.

The sexual reproductive strategy of *B. spicant* is mediated by an antheridiogen system, which could favor intergametophytic reproduction (Schneller *et al.*, 1990), and enhance genetic variability. Soltis and Soltis (1990) estimated low levels of selfing in several populations of *B. spicant*, consistent with the statement that fern species with antheridiogen systems are supposed to be effective outcrossers. Our molecular results, even in small populations, also indicate a moderate genetic diversity. *D. affinis*, on the contrary, presented low genetic diversity and it has an apomictic reproductive

cycle. Apomixis has been related to species of hybrid origin, allowing reproduction even in the presence of different genomes. This asexual reproduction also leads to an extreme genetic fixation in each population and without the possibility of sexual reproduction, a reduced genetic variability (Chao *et al.*, 2012). This is coincident with the extreme reduction of variability that we detected in each site sampled.

Our results confirm the importance of the reproductive system in determining the genetic diversity in fern populations. The apomictic fern *Dryopteris remota*, presents low genetic diversity, caused by the accumulation of somatic mutations (Schneller *et al.*, 1998). In the alloploid fern *Dryopteris cristata*, low genetic variability was also reported. In addition, a further reduction attributed to former bottlenecks was detected in populations with less than 25 individuals (Landergott *et al.*, 2001).

The genetic variability determines the response to environmental short and medium-term changes, allowing them to overtake stochastic factors that could lead to local extinction. Also, there are taxa that due to their complexity could be difficult to catalog by a species-based approach, leading to difficulties in the conservation efforts that should be focused on the conservation of the processes that lead to the generation of the biodiversity (Ennos *et al.*, 2012). One of the species that could be favored by a comprehensive approach in conservation actions is *D. affinis*. The *Dryopteris affinis* complex has different ploidy levels and reticulate origin of the subspecies (Fraser-Jenkins, 1980). Because the lack of sexual reproduction, each population has extremely local fixation of its low genetic variability, possibly generated by somaclonal mutational events *in situ*, even among populations sited in a geographically close area, making highly risky the introduction into a certain locality of individuals from other areas, even if they are close. On the other hand, the conservation of *Blechnum spicant*, an outcrossing fern species, might need different actions. Even in the small populations analyzed here, a relatively high genetic diversity was assessed. In addition, the Bayesian-clustering of our molecular data detected two groups among our samples, one including the locality B1 (Nueva) and other B2 (Novales) and B3 (Purón). B2 and B3 are sited less than 4 km away, so it is not that surprising that some genetic exchange is present between these two localities. On the contrary, Nueva is over 20 km away. The high variability detected suggests a continuous genetic flow among locations that once properly identified could be stimulated to recover threatened populations.

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Revealing a Cryptic Fern Distribution Through DNA Sequencing: *Pityrogramma trifoliata* in the Western Andes of Peru

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Abstract.—Fern identification usually requires the use of mature sporophytes, since attempts to identify juveniles using morphological traits often provides unsatisfactory results. Here we examined young sporophytes found among boulders in a river basin of a xeric valley in central Peru. Attempts to identify these sporophytes first pointed to four different genera, two in Pteridaceae (*Anogramma* and *Pityrogramma*), and the others in Aspleniaceae (*Asplenium*) and Cystopteridaceae (*Cystopteris*). Here, we resolved this puzzle combining morphology and sequences of DNA (*rbcL* and *trnG-R*) that point to *Pityrogramma trifoliata* of Pteridaceae.

Key Words.—Andes, DNA sequencing, *Pityrogramma*, young sporophytes

Species of the Neotropical fern flora are, in general, morphologically identifiable thanks to floristic treatments (e.g. Tryon, 1989–1994; Mickel and Smith, 2004; Smith et al., 2005) and recent revisionary studies (e.g. Link-Pérez and Hickey, 2011; Vasco, 2011). However, the nature of the fern life cycle, with its two independent phases, offers a challenge for identifiying gametophytes and juveniles sporophytes, since those floristic or taxonomic treatments rely on morphological characters of the mature, sporophytic phase. This study shows an example of how DNA sequencing techniques can expand our capabilities for identification. In this case, they also helped to confirm a surprising range extension.

Fern richness and species distribution in the Neotropics are associated with forested, wet locales. Indeed, in Peru, 70% of fern diversity is found in the eastern Andean slopes that overlook the Amazon. For the Pacific basin (both coastal and western Andes below 7° South), there is a range of xeric to mesic

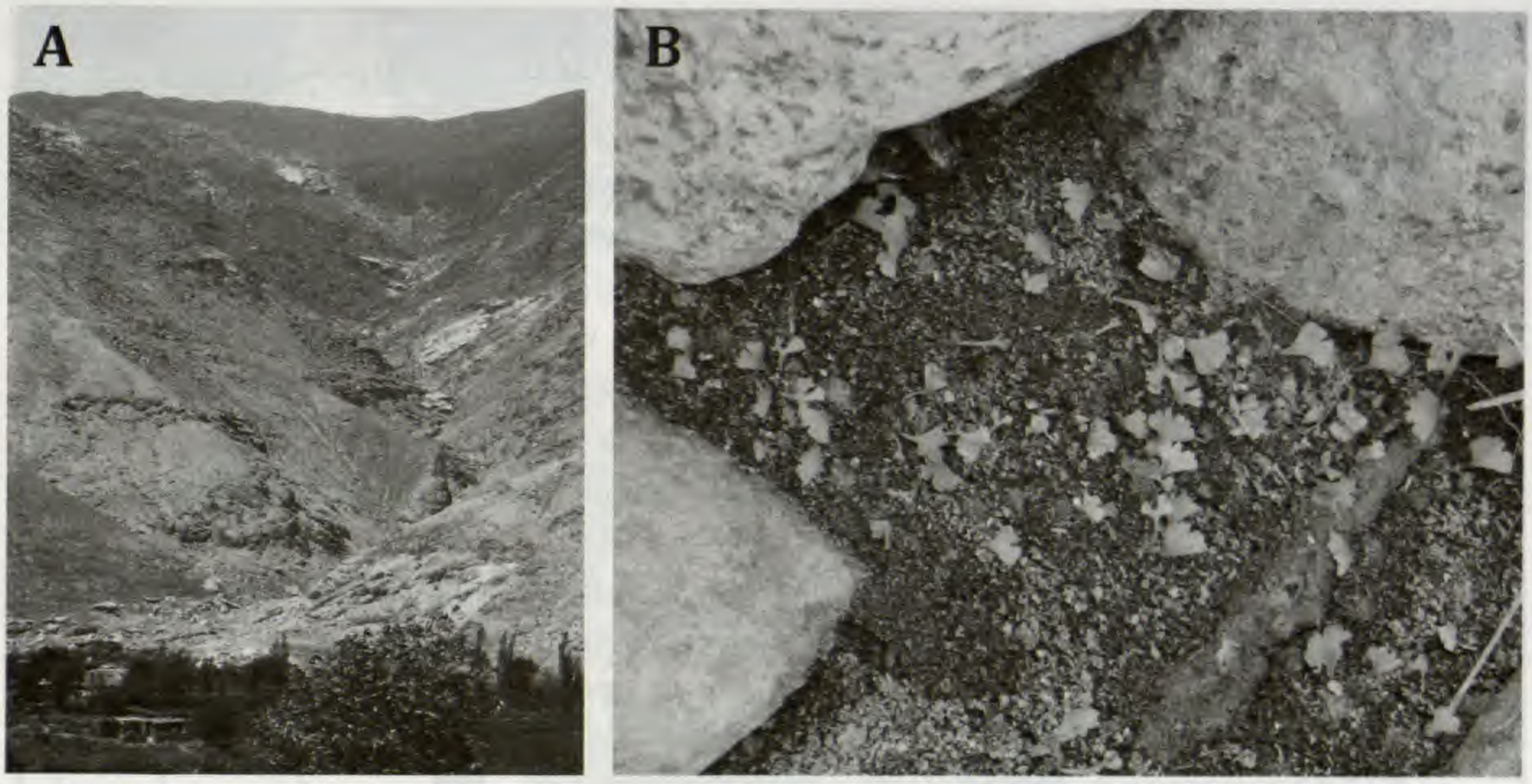


FIG. 1. Locality of the mystery fern in the valley of Mala, in central coastal Peru. A) View of barren hillslopes from the southern side of the valley at 400 m elevation. B) Gametophytes and young sporophytes under boulders near the Mala river.

environments along an altitudinal transect that harbors only 20% of the fern flora, usually occupying patchy habitats. An extreme xeric belt is known between 400 and 1000 m elevation in this basin.

Most members of the Peruvian Pacific basin fern flora belong to three families: Pteridaceae (dominated by cheilanthoids and pteroids *sensu* Schuettpelz et al., 2007; Rothfels, 2008), Aspleniaceae and Dryopteridaceae (León and Valencia, 1988; León and Young, 1999; León et al., 2002). All the coastal species are widely distributed and floristically they represent a subset of the western Andean fern flora (Tryon, 1960; León et al., 2002) comprising over 250 species.

In August 2011, a small population of gametophytes and young sporophytes was found under and among boulders along the river bed of a narrow xeric valley in central Peru, at 400 m elevation in a site with vegetation limited to the river margins (Fig. 1). These plants were initially assumed to belong to the common non-native *Adiantum capillus-veneris* L., which is found in nearby irrigation canals. However, the development of new leaves from material collected for cultivation, showed basal pinnae bearing acroscopic segments, so it was clearly another entity.

Some morphological characters such as indument and venation can aid in the identification of young sporophytes at the family and genus levels. Recently, molecular markers (DNA barcodes) have been used to improve our ability to identify problematic or easily confused ferns (e.g., Schneider and Schuettpelz, 2006; Li et al., 2009, 2011; Pryer et al., 2010; Yansura and Hoshizaki, 2012), and here this combined approach of morphological and molecular data was employed to resolve the identity of this puzzling Peruvian fern.

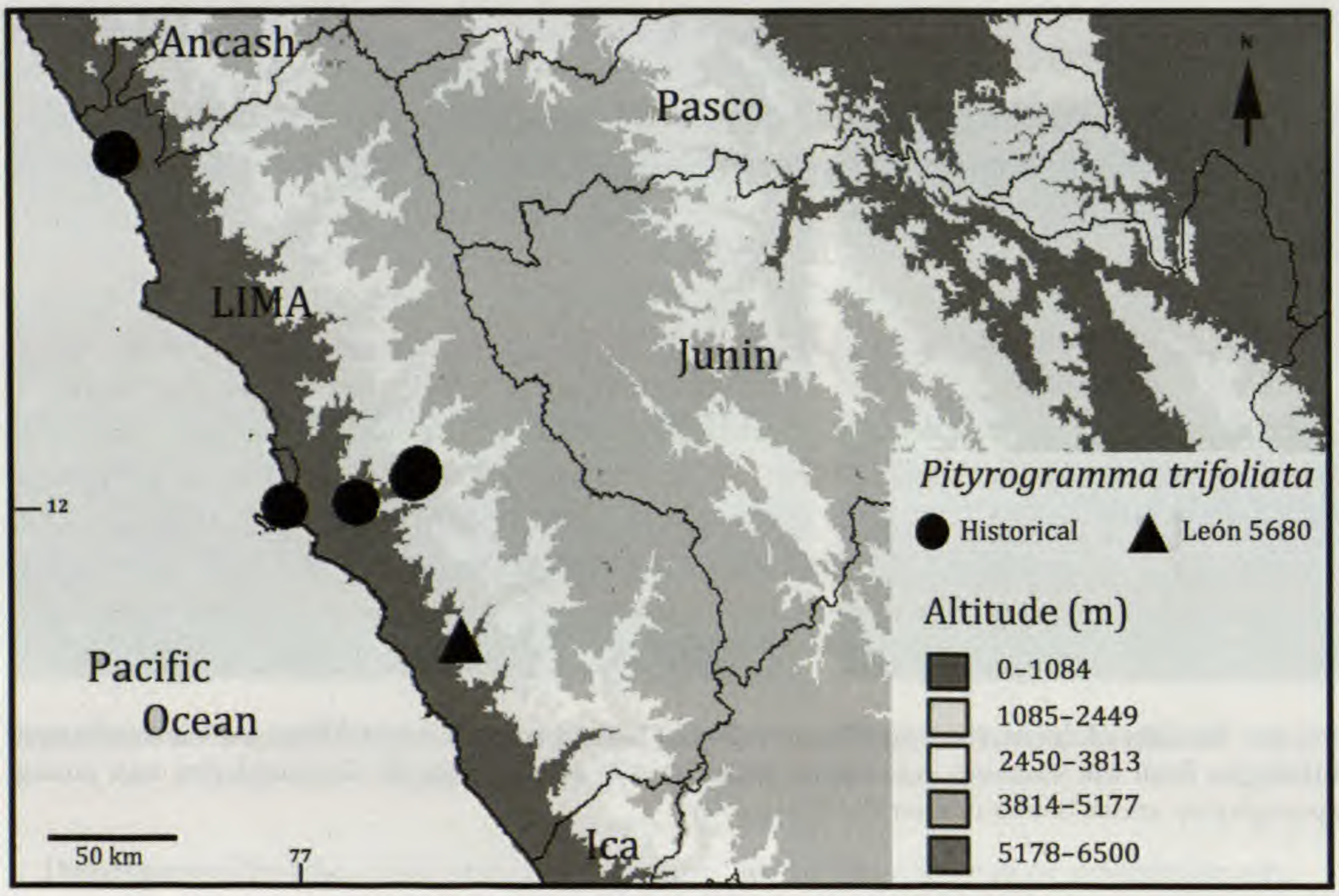


FIG. 2. Localities of all records of *Pityrogramma trifoliata* known from central-western Peru; circles represent historical records; triangle represents recent collection (B. León 5680, USM).

MATERIALS AND METHODS

Plants were obtained in the field, from a site in the Department of Lima, Province Cañete, in the Mala river basin, above the town of Calango (12°31′17.54″S, 76°30′1.11″W), at near 400 m elevation (Fig. 1A–B; Fig. 2), where climate conditions are hyper-arid (Rundel et al., 2007). They were present as small, undeveloped gametophytes and very young sporophytes. Plants and their surrounding soil were collected and maintained in a plastic-covered container for nearly 5 months, from August 2011 until February 2012. Two samples were taken in November 2011 to prepare herbarium vouchers and to extract DNA. Plants did not survive past February 2012.

DNA was isolated from dry tissue using the MP FastDNA® SPIN Kit and FastPrep® instrument (MP Biomedicals LLC, Solon, OH, USA).

Portions of two plastid loci—*rbcL* and the *trnG-trnR* intergenic spacer (henceforth, *trnG-R*)—in 21 μL reactions were amplified following established protocols (Rothfels et al. 2013). PCR was performed with an initial four-minute denaturation step (95°C), followed by 35 cycles of 30 seconds denaturation (95°C), 30 seconds elongation (40°C), and one minute elongation (71°C). The reaction was concluded with a final elongation step at 71°C, for 10 minutes. The *rbcL* amplifications used the primers *ESRBCL1F* and *ESRBCL654R* (Schuettpelz and Pryer, 2007), and the *trnG-R* reactions used *TRNG1F* and *TRNG63R* (Nagalingum et al., 2007). PCR products were purified using Shrimp

Alkaline Phosphatase (USB, Cleveland, Ohio) following established protocols (Rothfels et al., 2012) and sequenced on an ABI Prism 3700 DNA Analyzer (Applied Biosystems) at the Duke University Genome Sequencing and Analysis Core Resource, again using established protocols (Schuettpelz and Pryer, 2007). The forward and reverse chromatograms were assembled and edited in Sequencher 4.5 (Gene Codes Corporation), and the final sequences deposited in GenBank.

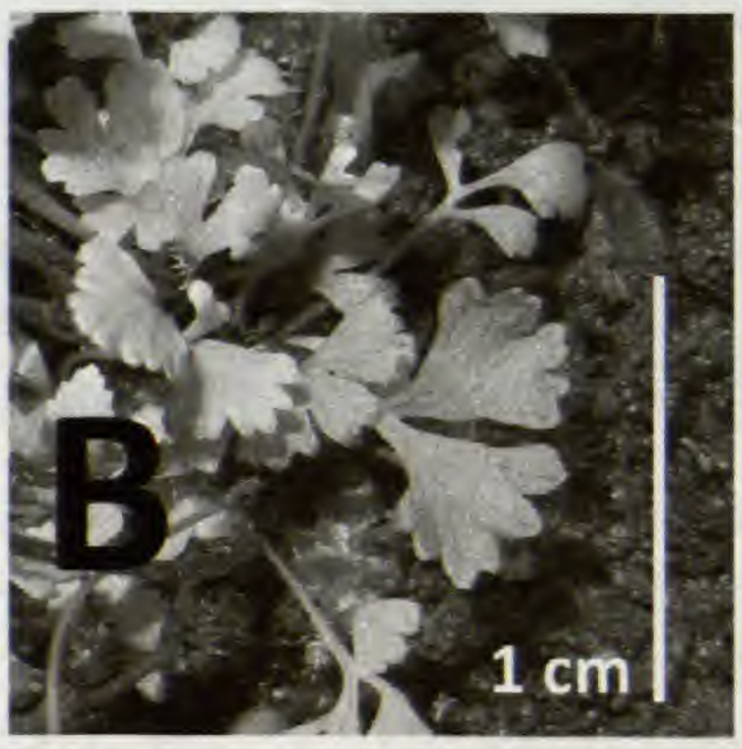
Based on the results of the preliminary BLAST comparisons (see below), we aligned the new sequences with the corresponding regions of previously published sequences spanning the Pteridaceae. For the *rbcL* alignment, we used sequences generated by Schuettpelz et al. (2007), with the exception of one sequence (AF336104), which is from Gastony and Johnson (2001). This *rbcL* dataset focuses on pteroids (*sensu* Schuettpelz et al., 2007; Rothfels, 2008) and involved no alignment issues (i.e., there were no indels). Fewer sequences were available for comparison with our *trnG-R* sequence. For this locus, we selected as wide a sample as possible, from Rothfels et al. (2008); it is primarly composed of cheilanthoids (*sensu* Schuettpelz et al., 2007; Rothfels, 2008), but includes one pteroid, and a cryptogrammoid. The sequences were aligned by hand, with ambiguous areas excluded prior to analysis. Heuristic tree searches on both datesets were performed under maximum parsimony in PAUP* v4.0A125 (Swofford, 2002).

Results

Gametophytes were oblong-cordate, nearly symmetrical, and glabrous. Most gametophytes were found in clumps in the field (Fig. 1B, 3A), and in this arrangement the lateral sides of the thalli were oblique to the midrib.

The initial sporophytic leaves were cuneate (Fig. 3A), while later, but still very young leaves had a pair of lateral segments similar in size to the apical one (Fig. 3B). In a month's time, we observed the development of pinnate-pinnatifid leaves (Fig. 3C), and these had a basal pair of pinnae with either an acroscopic segment, or segments along both the acroscopic and basiscopic sides (Fig. 3D). The leaves had an open venation that was 2–3 times furcate, and the apices of veins ended at the toothed margin (Fig. 3E); for young leaves, marginal teeth were not clearly developed. Well developed sporophytes also bore linear-lanceolate rhizome scales that were light brown, sometimes iridescent, and with elongate cells. In leaves with more than one pair of pinnae, indument consisted of multicellular, non-glandular hairs, initially sparse at the base of the rachis.

Attempting an identification based strictly on leaf morphology suggested the possibility of four different genera: *Anogramma*, *Asplenium*, *Cystopteris*, and *Pityrogramma*, all of which include taxa known in the local fern flora. Laminae that were once pinnate, with an acroscopic segment, pointed to a member of the Aspleniaceae (*Asplenium sessilifolium* Desv.) or Cystopteridaceae (*Cystopteris fragilis* (L.) Bernh.). The presence of non-clathrate rhizome scales eliminated *Asplenium*, but the presence of multicellular hairs similar to those



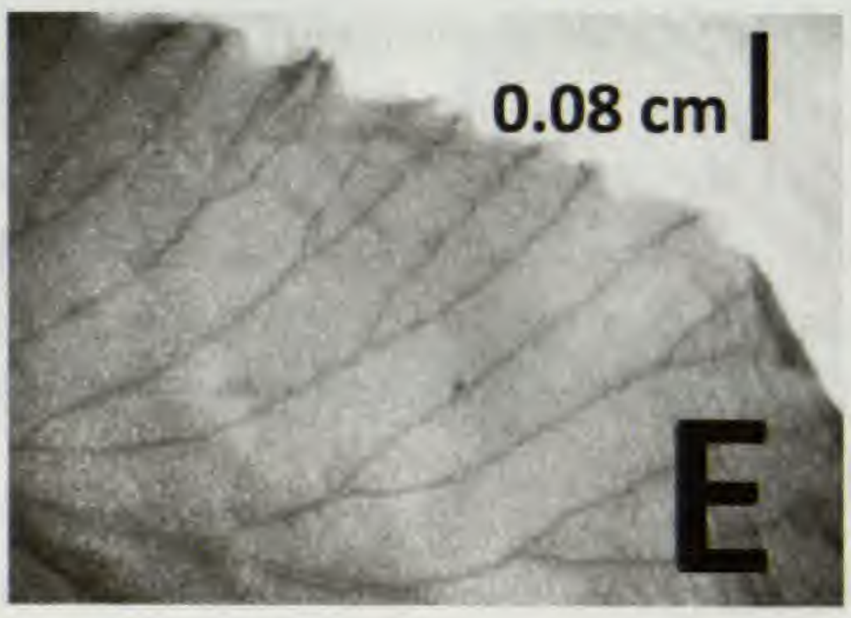


FIG. 3. A) Gametophytes and young sprorophytes showing furcate and pinnatisect laminae. B) Young sporophytes showing open venation. C) Four-month old sporophyte, fronds with elongate apical segments. D) Portion of pinnae, notice veins ending at tip of tooth. E) Young sporophyte, pinnae with acroscopic and basiscopic segments.

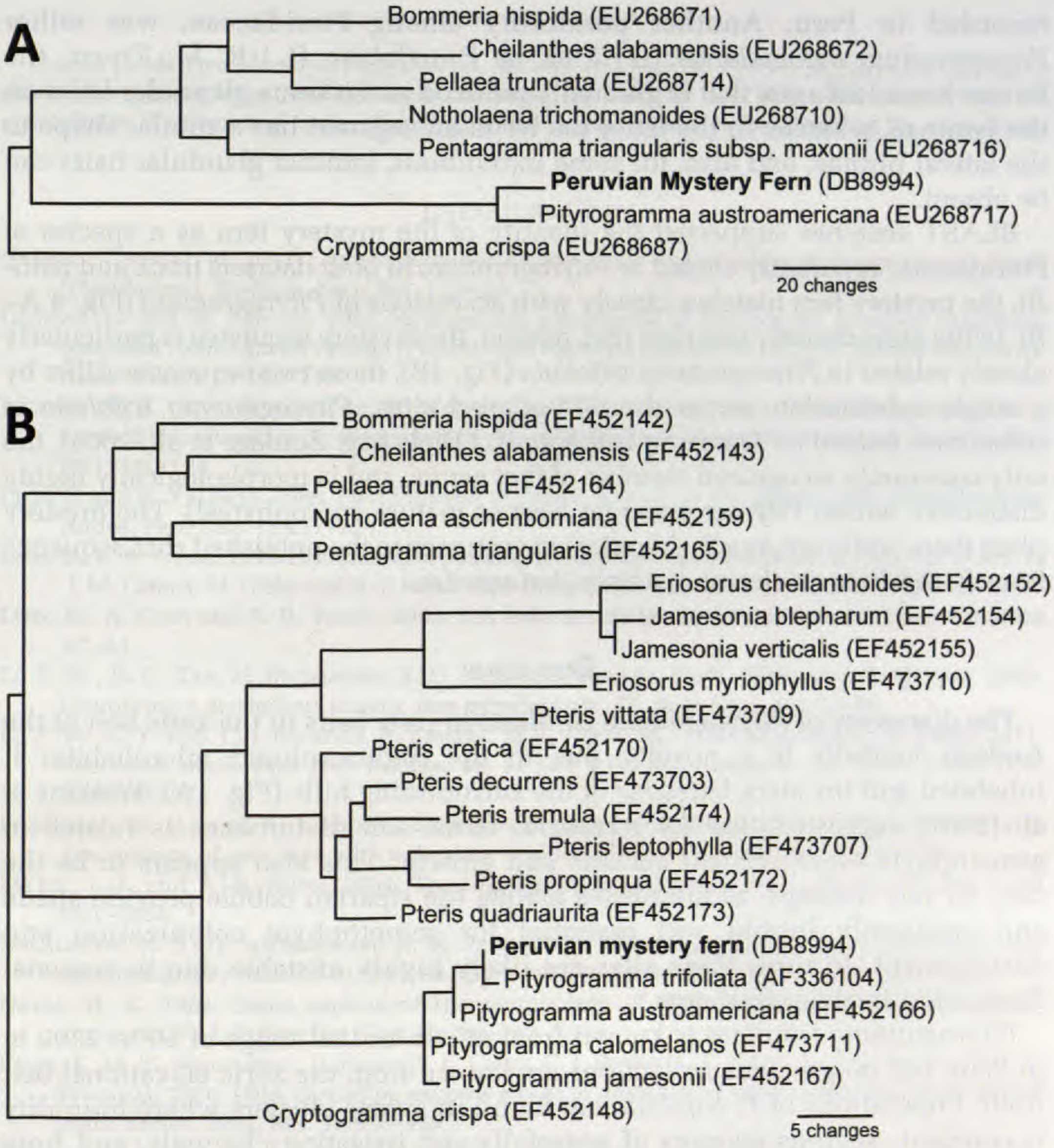


FIG. 4. Molecular phylogenies for the Peruvian mystery fern. A) The single most parsimonious tree for the *trnG-R* alignment, showing the position of the mystery fern among a broad Pteridaceae sample from Rothfels et al. 2008. B) One of 12 most parsimonious trees for the *rbcL* alignment, showing the position of the mystery fern among a broad Pteridaceae sample from Schuettpelz et al. 2007 (AF336104 is from Gastony and Johnson, 2001). Numbers in parentheses are GenBank accession numbers.

of *Cystopteris* supported the latter, as did the presence of toothed margins, although *Cystopteris fragilis* has veins ending in a sinus rather than in a tooth. A third possibility was a member of the Pteridaceae, either *Anogramma leptophylla* (L.) Link or *Pityrogramma chaerophylla* (Desv.) Domin, but in both these species the lamina is gradually reduced, and in the former, the rhizome does not bear scales, but hairs; additionally, the latter has not yet been

recorded in Peru. Another possibility among Pteridaceae, was either *Pityrogramma calomelanos* (L.) Link or *P. trifoliata* (L.) R. M. Tryon; the former has a leaf apex that is gradually reduced and it bears glandular hairs on the laminae, whereas in the latter the terminal segment has a similar shape to the lateral pinnae, and also, for some individuals, laminar glandular hairs can be absent.

BLAST searches supported the identity of the mystery fern as a species of Pteridaceae, apparently closest to *Pityrogramma*. In both datasets (*rbcL* and *trnG-R*), the mystery fern matches closely with accessions of *Pityrogramma* (Fig. 4 A–B). In the more densely sampled *rbcL* dataset, the mystery sequence is particularly closely related to *Pityrogramma trifoliata* (Fig. 4B); these two sequences differ by a single substitution across the 607 aligned sites. *Pityrogramma trifoliata* is sometimes treated as *Trismeria trifoliata* (L.) Diels (e.g. Zuloaga et al., 2008), the only commonly recognized member of that genus, and is morphologically highly distinctive within *Pityrogramma* (at least as mature sporophytes!). The mystery plant then, while not exactly identical in sequence to the published *rbcL* sequence from *P. trifoliata*, is almost certainly that species.

Discussion

The discovery of *Pityrogramma trifoliata* in river beds in the xeric belt of the Andean foothills is a novelty due to the underexplored microhabitat it inhabited and the stark bareness of the surrounding hills (Fig. 1A). Watkins et al. (2007) suggested that for terrestrial ferns, soil disturbance is related to gametophyte establishment success and growth. This also appears to be the case for our findings, as microsites among the riparian cobble provide shade and constantly humid soil essential for gametophyte colonization and development. In turn, these sites are likely highly unstable due to seasonal fluctuation in river discharge.

Pityrogramma trifoliata is known from an altitudinal range of 50 to 2300 m in Peru, but no previous collections are known from the xeric elevational belt itself. Populations of *P. trifoliata* are found in sparse clusters where humidity is constant, such as margins of waterfalls and irrigation channels, and from which a few collections of this species in western Peru have been previously reported. This species and other western Andes ferns are likely characterized by higher dispersability, resilience, and based on this study, the capacity to survive as a gametophyte.

If we had been restricted to using morphological data for identification, we would have required more time and cultivation efforts for the plants to express those morphological characters associated with *P. trifoliata*.

An important note as to the state of our knowledge of the fern flora is the scarcity of collections for ferns that are considered to be common. A more complete set of data on the natural history of ferns is also needed, especially those verifying and updating information on gametophytes, such as recent work within a phylogenetic framework in Pteridaceae (e.g. Gabriel y Galán, 2011; Johnson et al., 2012).

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American Fern Journal 103(1):49–52 (2013)

Shorter Notes

Occurrence of Critically Endangered Pteridophyte *Helminthostachys zeylanica* (L.) Hook. in Burachapori Wildlife Sanctuary, Northeast India.—Out of 12000 species of pteridophytes that have been recorded globally, around 1000 species including fern allies are distributed in the Indian subcontinent. *Helminthostachys zeylanica* (L.) Hooker (formerly *H. laciniata* Voigt), commonly known as '*kamraj*' or '*Tunjuk-langit*' or fruiting fern, is an endangered monotypic species that belong to Ophioglossaceae (Helminthostachyaceae) and is distributed in Asia and Australia (Chandra *et al.*, Taiwania 53:170–209. 2008). In the present study, we are reporting on *H. zeylanica* from Burachapori Wildlife Sanctuary (latitude 26° 30′ 32″ N to 26° 33′ 40″ N; longitude 92° 35′ 54″ E to 92° 46′ 07″ E; 75 m asl; 44.06 km^2 area) located on the south bank of the mighty river Brahmaputra in Sonitpur and Naogaon districts of Assam in north-eastern India (Fig. 1a). The major part of the sanctuary is covered by grassland with patches of wetlands, locally called '*beels*'. The sanctuary is deeply flooded during the monsoon (May to September) and dry in the winter (mid-November to February). Annual mean maximum and minimum temperature averaged 33°C and 16.7°C respectively, while mean annual precipitation was 2000 mm with more than 80% rainfall occurring during the monsoon. Occasional rains also occurred during winter.

Helminthostachys zeylanica is a perennial deciduous fern mostly occurring in moist, swampy, and shady places. It's height ranges from 30–45 cm with an underground creeping rhizome from which roots grow gregariously. Leaves are greenish, pinnate to sub-palmate and free-veined with irregular serrate margins (Fig. 1b). Spores are trilete, globose with coarse surfaces and fused baculae with delicate projections (Fig. 1c). During a regular field visit, as part of a major research project on 'wetlands and livelihoods' in the Brahmaputra plains, we came across a few patches of *Helminthostachys zeylanica* interspersed in the wetland riparian forests of Burachapori wildlife sanctuary. Root parts of *H. zeylanica* have medicinal importance (Benjamin and Manickam, J. Traditional Knowledge 6:611–618. 2007). *Helminthostachys zeylanica* plants reproduce through spores and vegetatively by means of an underground rhizome. During several field visits between March to November 2011, we observed that its growth in natural habitats was very slow. Even in an undisturbed area, no significant increase in number of individual plants was noticed. Instead, the number of individual plants decreased gradually in all the patches during successive field visits. It appears that expansion of the agricultural area in the southern and eastern part of the sanctuary, intensive cattle grazing, extraction of non-timber forest produce (NTFP) and other biotic factors pose huge threats to the survival and natural regeneration of *H. zeylanica*.

Soil analyses revealed the disturbance level and its significant impact on the population of *H. zeylanica* and on soil ecological parameters (Table 1).

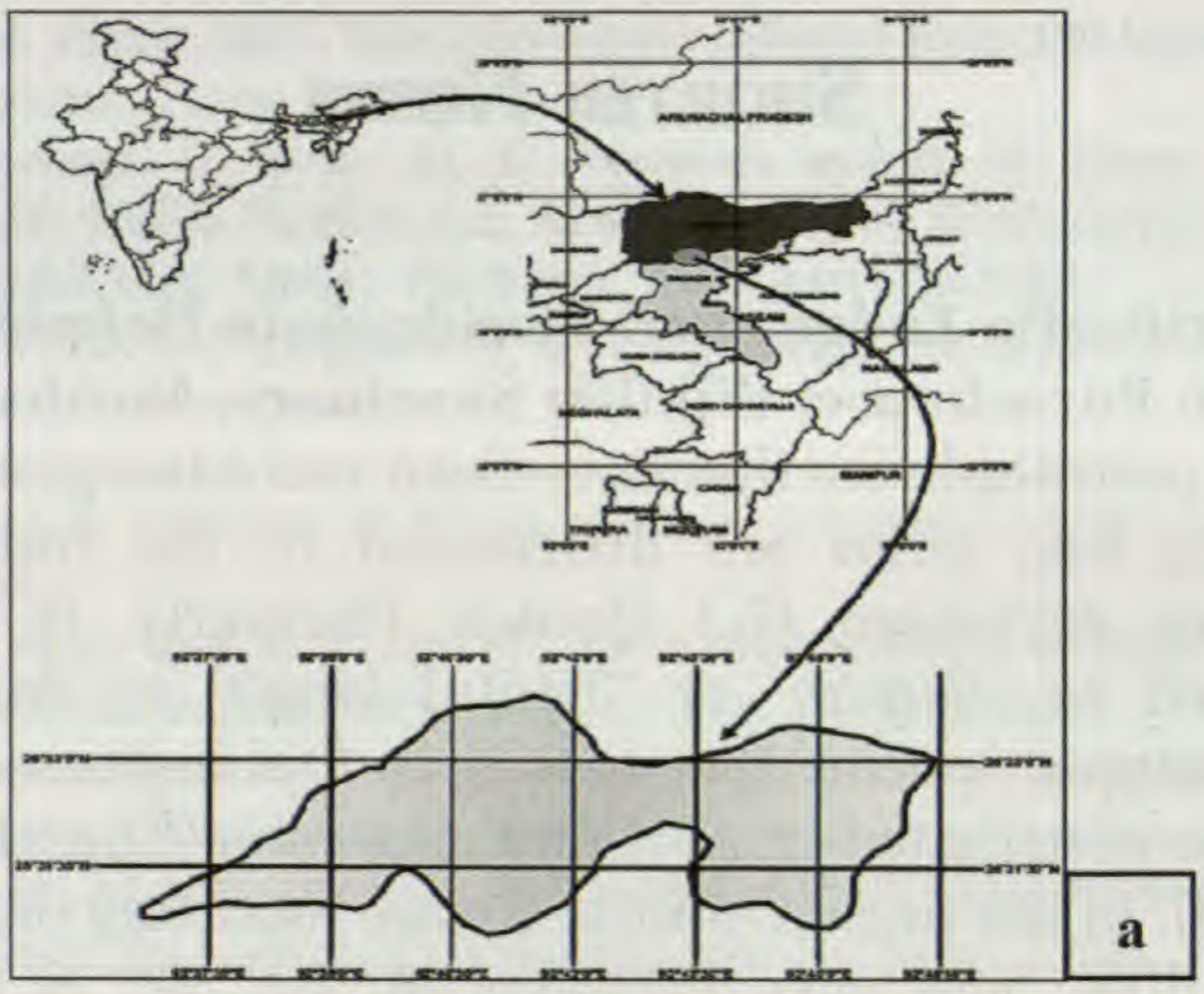

FIG. 1. Study site location (**a**) habitat (**b**), spores (**c**) of *H. zeylanica* and arrow points to the fertile spike with matured sporangium.

TABLE 1. Population structure, selected morphological characteristics of *H. zeylanica* and soil properties in Burachapori Wildlife Sanctuary, India.

Plant/Soil properties	Study sites				
	S1F	S2V	S3V	S4F	S5F
Population structure					
Patch density (Total leaves/m^2)	4.14 ± 0.31	2.40 ± 0.09	3.10 ± 0.11	4.33 ± 0.19	4.00 ± 0.24
Patch size (m^2)	7 ± 0.81	5 ± 0.44	10 ± 0.93	3 ± 0.02	6 ± 0.037
No. of Leaves					
Juvenile (m^2)	0.86 ± 0.001	0.80 ± 0.004	1.10 ± 0.03	0.67 ± 0.004	1.33 ± 0.05
Pre-Adult (m^2)	1.43 ± 0.008	0.60 ± 0.007	0.30 ± 0.001	2.33 ± 0.006	0.81 ± 0.002
Adult (m^2)	1.86 ± 0.03	1.00 ± 0.05	1.70 ± 0.02	1.33 ± 0.08	1.86 ± 0.09
Leaf morphology ($n=5$)					
Leaf height (cm)	39.13 ± 5.64	43.07 ± 7.89	40.55 ± 3.66	37.91 ± 6.20	45.38 ± 8.04
No. of leaflets	6 ± 0.34	4 ± 0.22	5 ± 0.21	5 ± 0.13	5 ± 0.34
Leaflet length	10.98 ± 3.09	9.21 ± 1.15	10.76 ± 2.98	13.93 ± 2.07	12.84 ± 2.78
Rhizome length (cm)	27.05 ± 4.80	24.86 ± 3.91	24.19 ± 5.06	30.02 ± 3.02	29.06 ± 1.83
Soil properties					
Texture	Sandy loam	Sandy	Sandy loam	Sandy loam	Sandy loam
Moisture (%)	39.06 ± 3.05	37.34 ± 2.98	37.21 ± 3.26	38.59 ± 4.06	38.77 ± 4.04
pH	4.32 ± 0.05	4.77 ± 0.02	4.89 ± 0.06	4.41 ± 0.03	4.43 ± 0.05
Organic matter (%)	5.36 ± 0.32	3.95 ± 0.59	4.52 ± 0.85	5.69 ± 0.53	5.26 ± 0.51
Total N (%)	0.56 ± 0.01	0.24 ± 0.03	0.49 ± 0.01	0.52 ± 0.01	0.41 ± 0.02
C/N ratio	5.55 ± 0.26	9.54 ± 0.85	6.78 ± 0.24	6.35 ± 0.46	7.44 ± 0.19
Available P ($\mu g\ g^{-1}$)	6.07 ± 0.35	4.91 ± 0.11	6.23 ± 0.36	5.87 ± 0.28	5.83 ± 0.27
K (ppm)	145.33 ± 12.94	138.02 ± 10.63	142.46 ± 9.20	142.30 ± 10.05	145.08 ± 9.14
Microbial biomass C ($\mu g\ g^{-1}$)	433.09 ± 55.09	282.37 ± 84.98	354.41 ± 57.86	369.01 ± 80.11	781.56 ± 105.06
Microbial biomass N ($\mu g\ g^{-1}$)	80.13 ± 15.33	52.27 ± 18.05	72.35 ± 11.97	75.41 ± 13.09	87.02 ± 20.03
Microbial biomass P ($\mu g\ g^{-1}$)	39.13 ± 5.09	24.92 ± 10.78	32.05 ± 9.11	40.09 ± 8.64	45.63 ± 7.05

S1F, S4F and S5F – Sites located in fringe or inside forest area; **S2V and S3V** – Sites located in fringes of villages ± S.E. (n=3)

Population density showed significant ($p<0.05$) positive correlations with soil moisture ($r=0.675$), pH ($r=0.849$) and organic matter content ($r=0.910$). Soil was generally acidic in all the patches (Table 1). Furthermore, soil microbial biomass C, N and P was greater in patches S1F, S4F and S5F in or near forest areas and lower in patches S2V and S3V near villages.

In this protected area, prescribed burning is a rotational phenomena that facilitates fresh growth of grass for grazing. However, burning also facilitates the growth of several dominant invasive species such as *Mimosa invisa* Mart., *Lantana camara* L., *Mikania* sp. and *Parthenium* sp. that potentially hinder the expansion of populations of *H. zeylanica* due to their physiological and reproductive advantages (Baker, Ann. Rev. Ecol. Syst. 5:1–24. 1974). It was interesting to learn that, in spite of its habitat destruction, the local people residing in and around the sanctuary were not aware of this fern's medicinal value. There was no written and/or published record of *Helminthostachys* in the biodiversity list available with the forest department. It was for the very first time that this endangered fern has been inventoried during our field visits.

Despite the ecological rarity of *H. zeylanica* and its intrinsic medicinal properties, this species has not been included in the conservation list of the Convention on International Trade in Endangered Species (CITES) of Wild Fauna and Flora, nor has it been listed in the prioritized medicinal plant list prepared by the National Medicinal Plants Board of India. Based on our survey and study of soil properties of the habitat of *H. zeylanica* we suggest that conservation policies that are largely sanctuary-specific and community-oriented will be the most effective. However, conservation of *H. zeylanica* by any *ex situ* conservation methods such as (a) integration in traditional agroforestry and/or (b) tissue culture regeneration may be challenging due to the unique environmental and physiological settings in which it grows.—D. Balasubramanian*, K. Arunachalam** & A. Arunachalam*. *Division of Natural Resource Management, Indian Council of Agricultural Research, Pusa, New Delhi-110012, India. **School of Environment and Natural Resources, Doon University, Dehradun 248001, Uttarakhand, India.

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Shorter Notes

The Identity of the Type of *Polypodium gamerianum* Vareschi (Polypodiaceae).—Volkmar Vareschi (Acta Bot. Venez. 1(2):117. 1966) described *Polypodium gamerianum* Vareschi, among 24 new species from Venezuela. Later, in his treatment of the ferns for the project "Flora of Venezuela" (Flora de Venezuela 1:915. 1969), Vareschi added no further information regarding this species; in fact, the description was exactly the same. In the intervening years, Vareschi prepared his flora using the system proposed by Copeland (*Genera Filicum*, Chronica Botanica Co. 1947), but in the case of Family Polypodiaceae Bercht. & C. Presl he used a broad concept for the genus *Polypodium* L., grouping several species of grammitid and polypod ferns, while putting aside other genera proposed by Copeland. This system differs drastically from that proposed by Morton (Contr. U.S. Natl. Herb. 38:85–89. 1967), and later by Tryon and Tryon (Ferns and allied plants with special reference to tropical America:684, 746. 1982), who included grammitid ferns as a tribe comprising a single genus *Grammitis sensu lato* within the Family Polypodiaceae. Consequently, Duek and Lellinger (Amer. Fern J. 68:120. 1978) made a new combination for *P. gamerianum*, based on a study of the type, *Vareschi & Mägdefrau 6839* (VEN; Fig. 1A), transferring this species to *Grammitis* Sw.: *Grammitis gameriana* (Vareschi) Duek & Lellinger.

A few years ago, when the author started his work with Venezuelan ferns along with A. R. Smith (University of California, Berkeley, USA), the subject of the affinities of *Grammitis gameriana* emerged: Was this species a polypod or a grammitid and to which of the several new segregates of *Grammitis sensu lato* did it belong? The large and heterogeneous genus *Grammitis*, in the traditional broad sense, is now commonly subdivided into several monophyletic genera using DNA sequence data and various morphological characters (e.g., Labiak *et al.*, Amer. J. Bot. 97:1354–1364. 2010; Ranker *et al.*, Taxon 53:415–428. 2004; Sundue, Syst. Bot. 35:716–729. 2010; Sundue *et al.*, Syst. Bot. 35:701–715. 2010). These and other studies have provided a better understanding of the phylogenetic relationships between the components of *Grammitis s.l.*, and they have necessitated recent nomenclatural changes.

Study of the type specimen of *P. gamerianum* deposited here in VEN reveals that this species has tetrahedral, trilete-scarred spores, but the green color typical of all grammitid spores has been lost due to the age, and the spores of the type are now white to yellowish. This observation, however, is enough to confirm the early assumption by Duek and Lellinger (1978) that this species is a grammitid. Moreover, the type specimen has free veins, entirely lacks setae on axes, laminae, and around the sori, and has abundant white or yellowish, wax-like glandular trichomes on both sides of the lamina, and also in the sori (paraphyses; Fig. 1B). The other feature observed is the absence of hydathodes on the adaxial surface of the laminae, which strongly suggests that *P. gamerianum* is related to the anhydathodous grammitid genera. In the

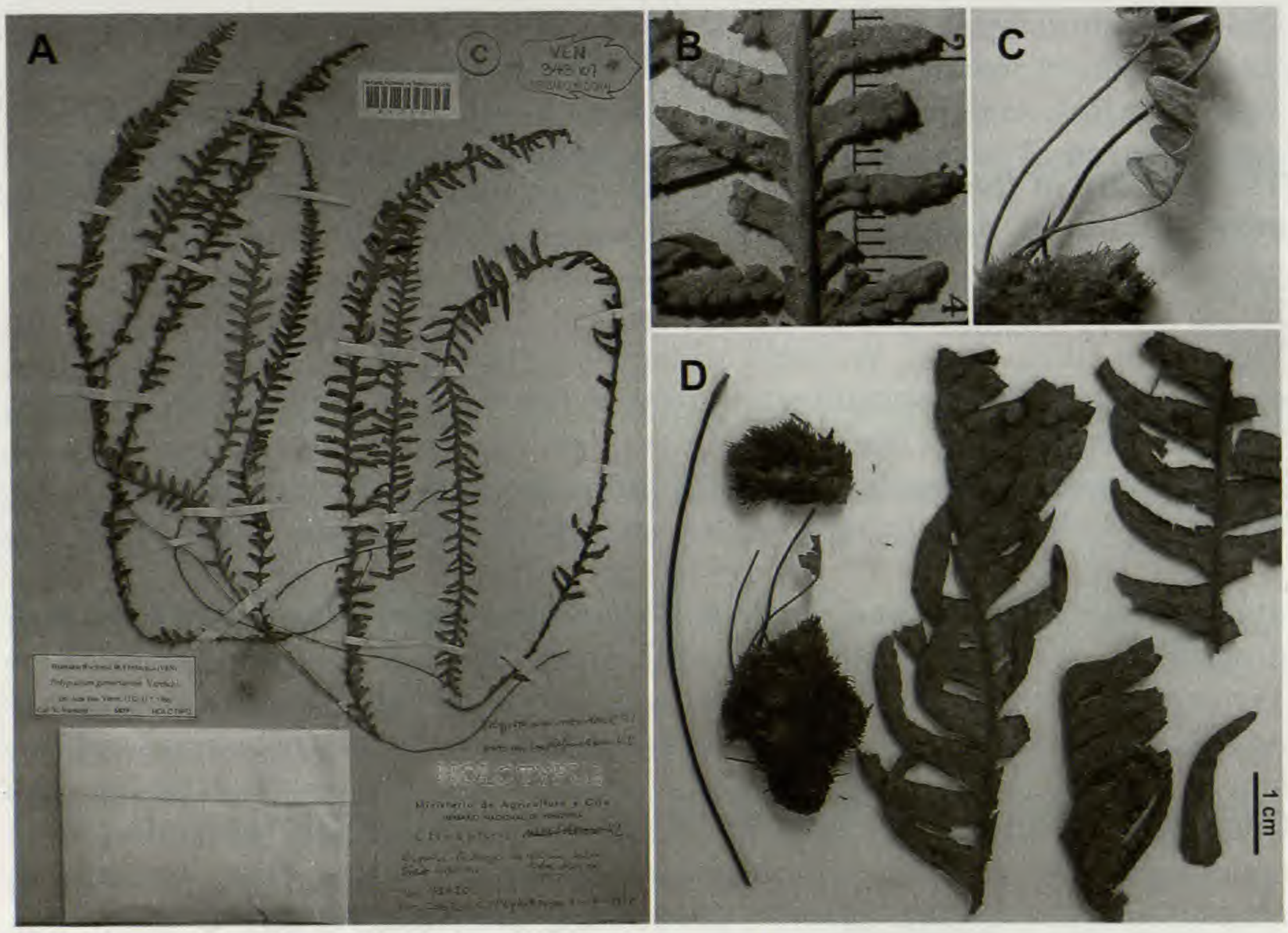

FIG. 1. A. Type specimen of *Polypodium gamerianum* Vareschi (*Vareschi & Mägdefrau 6839*, VEN); B. Abaxial surface of largest fronds; C. *Ceradenia intonsa*, mixed with *C. gameriana*; D. Fragments of *C. intonsa* which have been excluded, and contained in the packet placed in the lower left corner of type.

Neotropics, the anhydathodous genera are *Ceradenia* L. E. Bishop (Bishop, Amer. Fern J. 78:1–5. 1988), *Enterosora* Baker (Bishop and Smith, Syst. Bot. 17:345–362. 1992), and *Zygophlebia* L. E. Bishop (Bishop, Amer. Fern J. 79:103–118. 1989b), which form a monophyletic clade according to the most recent phylogenetic hypotheses (Ranker *et al.* 2004; Sundue *et al.* 2010). Nearly all other remaining Neotropical grammitid genera have adaxial hydathodes, except possibly a few species of the *Cochlidium* (A. R. Smith, com. pers.).

It is clear that *P. gamerianum* does not belong in *Enterosora* (which has laminar setae and lacks glandular paraphyses among the developing sporangia; Bishop and Smith, 1992). *Zygophlebia*, usually has areolate venation (regular anastamoses), and is also characterized by having glandular paraphyses, but these trichomes are brown, and thickly viscid, with sori adhering in a single sticky mass (Bishop, 1989b). In contrast, the type of *P. gamerianum* has larger fronds and a coriaceous texture, free veins, laminae covered with white to yellowish, sessile, spherical wax-like glandular hairs on both surfaces, and sori that have wax-like gland-bearing receptacular paraphyses. These last characters are typical in species of *Ceradenia* subg. *Ceradenia* (Bishop, 1988). Additionally, these gland-bearing paraphyses show no tendency to adhere to

each other or to the sporangia, as observed by Bishop (1989b) in *Ceradenia*, and by Rakotondrainibe and Deroin in *Zygophlebia* (Taxon 55:145–152. 2006).

Examination of the type of *P. gamerianum* shows that it should be placed in the genus *Ceradenia*. However, another problem arose in a more detailed examination of the type specimen. The morphology of the largest fronds on the sheet match the original diagnosis, but not the rhizome previously attached to the type (Fig. 1A; see the dark spot on the lower left, and below the eight largest fronds and retained in a packet). It does not belong with the eight fronds, and thus the type of *P. gamerianum* is a mixed collection with another species of the genus *Ceradenia* (Fig. 1C). The rhizome, which exhibits dorsiventral symmetry, is attached to a few fragmentary fronds, which have a herbaceous texture, and are provided on both sides of the laminae, rachis, costae, and margins with cylindrical, castaneous setae, 1.0–1.5 mm long. Furthermore, setae are numerous abaxially, and at the apex of petioles, they are intermixed with some branched hairs. Fronds lack spherical wax-like glandular hairs on both surfaces, but they do have white, wax-like gland-bearing paraphyses in the sori. Other features of this packet-fragment are: scales of rhizome dark brown, shiny, linear-lanceolate, to 6 mm long, ca. 0.6 mm wide, bearing on the margins abundant hyaline to yellowish setulae; petioles black to castaneous at apices, to 6.5 cm long; rachis sclerenchyma not exposed abaxially; pinnae 2 cm long, ca. 6 mm wide, basally sursumcurrent, margins entire to slightly crenate (when fertile), slightly auriculate acroscopically, little dilated basiscopically, and with apices rounded. The traits of the rhizome and the laminae suggest this other species is a member of *Ceradenia* subg. *Filicipecten* L. E. Bishop (Bishop, 1988), and was collected with and included in the original description (in part), accidentally mixed by Vareschi in the type specimen of *P. gamerianum*. The features described are consistent with the species currently known as *Ceradenia intonsa* L. E. Bishop ex A. León & Mostacero in León (Flora de Colombia 29:38. 2012). Another specimen of *C. intonsa*, *Vareschi & Mägdefrau 6836* (VEN) was collected from the same locality, suggesting that both species grow together, which possibly resulted in the mixed collection.

Because the rhizome does not belong to *P. gamerianum*, whose description is based entirely on the fronds that are in the sheet and not in the packet, it is necessary to designate a lectotype on the same specimen (McNeill *et al.*, International Code of Nomenclature for algae, fungi, and plants:Art. 9.14. 2012), excluding the rhizome and frond fragments contained in the packet (Fig. 1D). This is done simultaneously with the following new combination also required, and adding a more detailed description of the largest fronds, constituting the type:

Ceradenia gameriana (Vareschi) Mostacero, *comb. nov.*

Polypodium gamerianum Vareschi, *Acta Bot. Venez.* 1(2):117, f.16. 1966. Type: Venezuela. Mérida: Laguna Los Anteojos, selva del Pico Bolívar, [approx. 8°32′N, 71°03′W], 4100 m, 3 April 1958, *V. Vareschi & K. Mägdefrau 6839*

(lectotype VEN!, here designated, excluding the rhizome, leaf portions still attached to it, and other fragments of similar texture in the packet located in the lower left corner, and the fig. 16C on the original description; everything excluded corresponding to another species, *C. intonsa*).

Grammitis gameriana (Vareschi) Duek & Lellinger, *Amer. Fern J.* 68:120. 1978.

Plants epipetric; rhizomes unknown; fronds 28–52 cm long, determinate, monomorphic, apparently erect, lacking setae throughout, covered with minute wax-like glandular hairs on both surfaces, these more dense abaxially, to 0.2 mm long, sessile, adpressed, branched, comprising 3–4 spherical glands less than 0.1 mm at the apex, appearing white to pale yellow; petioles 8–20 cm × ca. 0.5 mm, brown-stramineous, dull, glabrous; laminae coriaceous, 1-pinnatifid to -pinnatisect, narrowly linear, gradually reduced proximally, 21–50 × 1.2–3.5 cm, with 50–80 pairs of pinnae; rachis sclerenchyma slightly exposed and rounded abaxially, darkened, ca. 1 mm wide; pinnae 8–17 × 0.15–0.3 mm, set 45–90° to the rachis, each spaced 1–2 times its width, linear-lanceolate, oblong to linear, the bases decurrent basiscopically, straight or nearly so acroscopically, pinna margins entire (sterile) to undulate or slightly crenate (fertile), the apices rounded (sterile) to subacute (fertile); veins free, pinnate, branched once, not visible without transmitted light; hydathodes lacking adaxially; sori brown, round, 8–12(–18) per pinna, medial to submarginal, 0.8–1.2 mm wide, with wax-like gland-bearing receptacular paraphyses, these whitish, like those elsewhere on the fronds; sporangia not setose; spores tetrahedral, with trilete scars.

Ceradenia gameriana is known only from the type. It grows in paramos, at 4100 m, on mossy rocks. It appears to be more closely related to a group of mostly of terrestrial species growing in paramos, e.g., *C. herrerae* (Copel.) L. E. Bishop, *C. terrestris* L. E. Bishop, and *C. maxoniana* L. E. Bishop. The type of *C. maxoniana* (Lehmann 2400, from Dept. Tolima, Colombia, holotype US!, isotype B!, both online photo) with erect fronds is very similar, and it may prove to be the same species or conspecific (a possible later synonym), judging by the description (Bishop, Amer. Fern J. 79:24, Fig. 2D. 1989a). At first glance *C. gameriana* differs from all known species of *Ceradenia* by having a greater stature and longer petioles, characters that can be phenotypically plastic. The rhizome of *C. gameriana* is unknown, but presumably radially symmetrical (typical of subg. *Ceradenia*). More complete collections from the type locality are necessary in order to reach a better understanding on the subject of the relationships.

I thank A. R. Smith for doing a critical reading of the manuscript and helping me with the English grammar, as well as anonymous reviewers for their useful comments regarding this work.—Julián Mostacero Giannangeli, Universidad Central de Venezuela, Fundación Instituto Botánico de Venezuela, Herbario Nacional de Venezuela (VEN), Apartado 2156, Caracas1010-A, Venezuela, e-mail: julian.mostacero@ucv.ve.

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Review

Ferns of Southern Africa: A Comprehensive Guide, by Neil R. Crouch, Ronell R. Klopper, John E. Burrows, and Sandra M. Burrows. Struk Nature, Capetown, South Africa. 2011 (actually published on 12 January 2012). Price: $50.00 (available on Amazon.com) ISBN 978-1-77007-910-6 (for the softcover edition).

Enthusiasts of South Africa's diverse fern flora have been blessed with a number of exceptional publications over more than a century. In recent decades, in addition to the *Flora of South Africa* volume on Pteridophyta (Schelpe and Anthony, 1986), Werner B.G. Jacobsen (1983) set a very high standard with *The Ferns and Fern Allies of Southern Africa*, a large volume that was monographic in its level of detail and was illustrated with black-and-white photographs. John and Sandra Burrows (1990) extended this tradition with another large-format volume, *Southern African Ferns and Fern Allies*, equally detailed in its presentation and with the added advantage of color photographs and beautiful line drawings. Both of these have gone out of print in recent years, and there also has been a great deal of floristic and taxonomic research on ferns of the region during the past two decades, creating the need for an updated pteridoflora for South Africa.

The new book is smaller in its format than its predecessors, but is even more impressive in its content. Because the publisher is an imprint of the South African arm of publishing giant Random House, the book is both readily available internationally and very reasonably priced. The softcover edition is sturdily bound and has a weatherproof cover. Originally, limited numbers of copies were printed in hardcover and leatherbound editions, but these both were sold out before the book ever appeared.

The authors have a wealth of experience with South African ferns and lycophytes, and their experience shows in the precise descriptions and useful discussions, especially those based on their firsthand knowledge of ecological relationships of the species. The number of accepted taxa is 321 for the region, down from 343 in the earlier volume by Burrows because of extensive taxonomic revisions. Despite this, during the research six new taxa were discovered in the genera *Cheilanthes, Isoetes, Ophioglossum, Pilularia*, and *Selaginella*. These are formally described in a section on Taxonomic Notes toward the conclusion of the volume, in addition to their treatment in the main body of the book. The familial classification more or less follows that in the recent *Synopsis of the Lycopodiophyta and Pteridophyta of Africa, Madagascar, and Neighbouring Islands* (Roux, 2009), as do most of the generic and species circumscriptions.

Genus and species determinations are accomplished using dichotomous keys that are nicely set apart from the text with colored backgrounds, as well as tables differentiating closely related taxa. Throughout the book, from tables to maps to keys, there has been excellent use made of color in the layout. The key to families is an unusual construct consisting of a lengthy illustrated table that

differentiates the familial groups by first requiring a choice among multiple combinations of character states for life form, leaf type, and placement of sporangia, followed by further discrimination among clusters of families using other characters. The treatment of each species is broken into sections for synonymy, derivation of the epithet, morphological description, discussion of similar species, distribution, and a section on ecology and notes. Neophytes will find good use in an illustrated glossary and those seeking further technical information will appreciate the relatively inclusive bibliography (which includes the references cited in this review and many other important works).

For the new book, the authors decided to attempt to illustrate all of the species and their habitats with a new series of color photographs (more than 1,800 in total), and for many of the plants there are supplementary line drawings to highlight important details or otherwise amplify features. The authors were personally able to study all but two of the rarest species in the field. Several photographers provided images, but the Acknowledgements section notes that many of the new photos were taken by Arrie Klopper, whose beautiful images are very well-composed and especially useful for identification. The images apparently were selected from a body of about 30,000 photographs assembled for the project. Sandra Burrows provided the exquisite pen-and-ink drawings. One of the outstanding aspects of the book is the habitat photos, which give the reader a strong impression of where these plants grow. For most species, there is a series of images illustrating the habitat, whole plant, and details.

Ferns of Southern Africa is small enough to fit into a daypack and accessible to those just starting in ferndom, but is detailed and scientifically rigorous enough to stand as a monograph of the region's ferns and lycophytes. It belongs in the library of every South African botanist and fern enthusiast, and will be of interest to pteridologists elsewhere in the world. It is an outstanding example of a regional pteridoflora.—GEORGE YATSKIEVYCH, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166.

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Current Status of the Ferns and Lycophytes of the Hawaiian Islands

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ABSTRACT.—The Hawaiian Islands are well known for having one of the highest documented percentages of endemic plants in the world. Hawaiian ferns and lycophytes represent a relatively large percentage of the endemic flora with approximately 74% of the native fern and lycophyte species considered endemic. In addition, at least 40 taxa are naturalized aliens. We present a new synopsis of the Hawaiian fern and lycophyte flora that includes new state and island records, recent taxonomic updates and problems, and a summary of known and hypothesized geographical origins of fern and lycophyte lineages. We also provide a checklist and access to an interactive key to the native and naturalized ferns and lycophytes of the Hawaiian Islands (http://www.herbarium.hawaii.edu/lucid/ferns/introduction.html).

KEY WORDS.—Hawaii, fern, lycophyte, checklist, interactive key

There are 159 native species of ferns and lycophytes in the Hawaiian Islands. Although these represent only about 15% of the native vascular plant flora of the islands (159 fern and lycophyte species: 1032 flowering plant species) (W. L. Wagner, pers. comm. and W. L. Wagner *et al.*, 2005–), fern species are often common components of the vegetation of many mesic to wet plant communities and are sometimes the dominant species both in numbers of individuals and in total biomass (e.g., see Gagné and Cuddihy, 1999). In addition to the native fern and lycophyte taxa, there are now 40 introduced and naturalized taxa, some of which are becoming invasive. Thus, an accurate knowledge of fern and lycophyte diversity in the Hawaiian Islands is important for both basic ecological and evolutionary studies and for conservation management purposes. During the course of preparing an interactive, online key to the ferns of the Hawaiian Islands (see: http://www.herbarium.hawaii.edu/lucid/ferns/introduction.html), we conducted a thorough review and analysis of the taxonomic history of Hawaiian ferns and lycophytes. We present here a summary of those studies, including the history of collecting, an overview of fern and lycophyte diversity in the islands, estimates of the geographical origins of native Hawaiian fern and lycophyte lineages, additions to the known flora since the publication of the last floristic inventory (Palmer, 2003), and recent taxonomic changes.

Synopsis of Hawaiian Fern and Lycophyte Floras and Enumerations

It was upon Captain James Cook's voyage of the *HMS Resolution* in 1778 that the first collections of Hawaiian plants were made by the naturalist William Anderson (W. L. Wagner *et al.*, 1999). Anderson made a single collection,

Argemone glauca Pope, and noted 22 plant taxa occurring on the island of Kaua'i. The following year, David Nelson, an apprentice botanist aboard Cook's *Resolution* sister ship the *Discovery*, collected approximately 136 vascular plant specimens (St. John, 1978; W. L. Wagner *et al.*, 1999). Although the ship made excursions to Ni'ihau, Kaua'i, Maui, and Hawai'i, Nelson was able to explore only the island of Hawai'i. The specimens from these voyages are housed at the Natural History Museum in London. Archibald Menzies and Albert Chamisso also were early collectors of Hawaiian plants. Menzies explored five of the Hawaiian Islands from 1788 to 1794 (W. L. Wagner *et al.*, 1999). Chamisso collected in the 1810s on O'ahu and later published species descriptions in the journal *Linnaea* (Robinson, 1912a).

Charles Gaudichaud was the botanist aboard Louis de Freycinet's voyage of the *Uranie* that explored O'ahu, Maui, and Hawai'i for twenty days in 1819, although only seven days were spent on land (St. John and Titcomb, 1983). Gaudichaud collected 4,175 specimens throughout the journey (1817–1820), but unfortunately 2,500 of those were lost in a shipwreck. In 1826, he published his account of Hawaiian ferns and lycophytes in *Botanique du Voyage autour du monde* (Gaudichaud, 1826). He included descriptions for 52 genera and 268 species of ferns and lycophytes. Of these, 17 genera and 37 species were from the Hawaiian Islands.

Between 1825 and 1834, James Macrae, George T. Lay, and David Douglas made collections of Hawaiian ferns and lycophytes (Robinson, 1912a; W. L. Wagner *et al.*, 1999). The botanist William Brackenridge visited the Hawaiian Islands from 1840–1841, as part of the United States Exploring Expedition under the command of Charles Wilkes (W. L. Wagner *et al.*, 1999). Throughout the expedition, he recorded numerous ferns and lycophytes and published brief descriptions for all species, and he also included 46 plates with detailed illustrations (Brackenridge, 1971). Unfortunately the majority of the copies of this publication were destroyed in a fire and only 12 copies were salvaged (Robinson, 1912a). Images of Brackenridge's plates may be viewed at the Smithsonian Institution Libraries website: http://www.sil.si.edu/imagegalaxy/imageGalaxy_MoreImages.cfm?book_id=19-24a.

Several enumerations and diagnostic keys of Hawaiian ferns were made between 1864 and 1887. William T. Brigham and Horace Mann visited Kaua'i, O'ahu, Maui, Moloka'i, and Hawai'i and made a checklist of 113 fern species (Robinson, 1912a). John M. Lydgate (1873) published a synopsis of Hawaiian ferns that included descriptions of 24 genera and keys to 111 taxa. Derby (1875) published a checklist that included 124 fern and 15 lycophyte taxa. Several years later, Edward Bailey (1883) wrote a summary of the Hawaiian ferns that included 149 species, each with descriptions and several with elevational and habitat data, although no identification keys were included. Lorenzo G. Yates (1887) published an enumeration of Hawaiian ferns (129 species) that lacked species descriptions, but which incorporated some habitat and elevational information for most species.

William Hillebrand (1888) spent 20 years in the Hawaiian Islands working as a physician and developing his skills as a botanist. At the time of his death in

1886, part of his manuscript of "The Flora of the Hawaiian Islands" was with the printer. It was not until 1888 that the flora was published. The flora contains generic and specific keys and detailed descriptions for flowering plants, ferns, and lycophytes, including distributional and ethnobotanical information. Hillebrand recognized 29 fern genera with 140 species and two lycophyte genera with 15 species. He also acknowledged numerous varieties (approximately 50 fern and four lycophyte varieties) with the majority occurring within *Asplenium* species.

In 1890, A. B. Lyons created a diagnostic key to the Hawaiian ferns that included a key to genera and species, but he did not include species descriptions. Lyons included 27 genera and 143 taxa (139 species, three varieties, and one undetermined species) (Lyons, 1890). Seven years later, Amos A. Heller (1897) published a report of his observations of plants of the Hawaiian Islands. He provided brief descriptions for 106 fern and lycophyte taxa, including six newly described species, and illustrations for two species. Winifred J. Robinson published four papers over the course of three years (1912–1914) that included keys and detailed descriptions to the families, genera, and species of Hawaiian ferns and lycophytes. She recognized more genera and species than any previous treatment: 43 genera and 166 species of ferns and lycophytes (Robinson, 1912a, 1912b, 1913, 1914). Her treatment included numerous plates of photographs of herbarium specimens along with species descriptions that included synonyms, type localities, distribution, and ethnobotanical uses. Copeland published two papers in which he described several new species and updated the nomenclature for fern collections he received from M. L'abbé Faurie (1914) and Joseph F. Rock (1916).

Several years later, Vaughan MacCaughey focused on the ecological relationships of Hawaiian ferns and lycophytes, and recognized 44 genera and 190 species (MacCaughey, 1918). Carl Christensen revised the nomenclature of the Hawaiian ferns and lycophytes to follow his *Index Filicum* (1906). He published a checklist that accepted 41 genera with 159 fern and lycophyte taxa (Christensen, 1925). Although he did not include identification keys, he did provide additional notes on some species and groups that are in need of further study.

Robert L. Fowler (1940) created an annotated checklist of the ferns and lycophytes of the Kilauea-Mauna Loa section of Hawai'i Volcanoes National Park. His list included six lycophyte species and 60 fern taxa; some taxa included information on where they could be located within the park. In 1952, Douglass H. Hubbard created a booklet of the most common ferns of Hawai'i National Park that included images for 63 species and brief descriptions of the genera. From 1932 to 1980, Otto and Isa Degener published a series of loose-leaf books on the Hawaiian vascular plant flora (Degener, 1932–1980). Their publications include generic and species descriptions and keys, distributional information, and numerous illustrations for 94 fern and 14 lycophyte taxa. During the 1980s Warren H. Wagner Jr. published two papers in the *Fiddlehead Forum* that briefly described the status of the Hawaiian ferns and lycophytes, based on years of field experience by himself and Florence

S. Wagner. In 1981, he presented a checklist of approximately 180 fern and lycophyte taxa (W. H. Wagner, 1981). Seven years later he reported 198 taxa, 172 native (118 endemic) and 26 naturalized, although he acknowledged uncertainties about the exact total number of taxa (W. H. Wagner, 1988).

The two most recent Hawaiian fern and lycophyte floras are from Kathy Valier (1995) and Daniel D. Palmer (2003). Valier did not treat all the Hawaiian ferns and lycophytes, but rather provided descriptions for 61 of the most commonly encountered taxa. She reported, in accordance with W. H. Wagner (1988), about 200 fern and lycophyte taxa, 172 being native. The most up-to-date and comprehensive Hawaiian fern and lycophyte flora is that of Palmer (2003), which included descriptions of fern and lycophyte families, genera, and species along with keys to genera and species and numerous illustrations. Palmer recognized 73 genera and 221 taxa (including hybrids) of Hawaiian ferns and lycophytes. The species descriptions highlight characters that separate each species from similar species and include information on synonyms, current distribution, habitat, elevation, ethnobotanical knowledge, and vernacular names. Palmer was able to record the variability found throughout Hawaiian ferns and lycophytes by observing numerous specimens in the field and in herbaria and including first-hand observations of many type specimens.

Current Treatment

The Hawaiian Islands have representatives from all major lineages of monilophytes except the Equisetopsida and from all three lineages of lycophytes. The endemic fern genera of the Hawaiian Islands have long been thought to consist of *Adenophorus* (Polypodiaceae), *Diellia* (Aspleniaceae), and *Sadleria* (Blechnaceae). [N.B. Authorities for all Hawaiian taxa are given in Appendix 1.] However, molecular work by Schneider *et al.* (2004a, 2005) supported *Diellia* as a monophyletic clade nested within *Asplenium* (Aspleniaceae), therefore decreasing the number of endemic fern genera to two. This classification is recognized in the majority of recent treatments (Christenhusz *et al.*, 2011; Smith *et al.*, 2006, 2008; Snow *et al.*, 2011; Viane and Reichstein, 1991). There are presently 144 native fern species (167 taxa) and 15 native lycophyte species (16 taxa), not including hybrids in the Hawaiian Islands. The genera with the most Hawaiian fern taxa are *Asplenium* (29 taxa), *Dryopteris* (20 taxa), and *Adenophorus* (12 taxa). A synopsis of the statistics of the Hawaiian fern and lycophyte flora is given in Table 1.

New Fern Records in the Hawaiian Islands

Several new state and island records of native and naturalized ferns have been published since Palmer's flora (2003), including seven new state records of naturalized ferns (Table 2). In addition, 20 new island records have been reported, most of which are also naturalized ferns. One new island record of a native species is of the endemic *Asplenium haleakalense*, which was

TABLE 1. Current statistics for the ferns and lycophytes of the Hawaiian Islands[1].

	Ferns	Lycophytes	Total ferns and lycophytes
Families	25	3	28
Endemic genera	2	0	2
Genera with all naturalized species	16	0	16
Indigenous genera	48	5	53
Native genera	50	5	55
Total genera	66	5	71
Endemic species	110	7	117
Indigenous species	34	8	42
Total native species	144	15	159
Endemic hybrids	18	6	24
Indigenous hybrids	0	0	0
Total native hybrids	18	6	24
Endemic taxa *	132	8	140
Indigenous taxa *	35	8	43
Total native taxa *	167	16	183
Naturalized species	33	3	36
Naturalized taxa *	37	3	40
Total native and naturalized species	177	18	195
Total native and naturalized taxa *	204	19	223
Total native and naturalized taxa **	222	25	247

[1] includes species presumed extinct
* includes subspecies, varieties, and cultivars, but does not include forms or hybrids
** includes subspecies, varieties, cultivars, and hybrids

described in 1999 and known previously only from Maui (Palmer, 2003). A specimen at the Herbarium Pacificum (BISH) collected on Hawai'i in 1995 was determined as *A. haleakalense* by Dan Palmer in 2002, thus representing a new island record for this species (Kennedy *et al.*, 2010). *Microlepia speluncae* (Dennstaedtiaceae) is another example of a new island record for a native species. Palmer (2003) reported the species to be distributed on the islands of Kaua'i, O'ahu, and Hawai'i, but it has been documented subsequently on Maui (Oppenheimer, 2003) and Moloka'i (Oppenheimer, 2008).

Four noteworthy rediscoveries include *Asplenium diellaciniatum* (syn. *Diellia laciniata*), *Asplenium dielmannii* (syn. *Diellia mannii*), *Ctenitis squamigera* (Dryopteridaceae), and *Pteris lidgatei* (Pteridaceae) (Table 2). *Asplenium diellaciniatum* was rediscovered on the island of Kaua'i (Wood, 2006). Palmer (2003) recognized this taxon as a form of the federally listed species, *Diellia erecta* (f. *alexandri*), and noted it was likely extinct on Kaua'i. Recent morphological studies by Lorence et al. (in press) conclude that *Diellia erecta* f. *alexandri* represents two distinct species with one endemic to Kaua'i (*Asplenium diellaciniatum*) and the other to Maui Nui. In 2004, a population of 49 individuals was discovered on slopes of the Kawai'iki Stream in western Kaua'i (Wood, 2006). The most recent report documents a decline in the population to 39 individuals (USFWS, 2009a). Currently the species is not

TABLE 2. New species, state records, island records, and rediscoveries of ferns and lycophytes in the Hawaiian Islands subsequent to Palmer (2003).

Taxon	Island								Reference
	N	K	O	Mo	L	Ka	Ma	H	
Adiantum 'Edwinii' [NZ]			**I**		x		x		Lau and Frohlich, 2012
Angiopteris evecta [NZ]		**I**	x	**I**	x		x	x	Oppenheimer, 2010; Wood, 2006
Asplenium diellaciniatum [N]		**D**							Wood, 2006
Asplenium dielmannii[N]		**D**							Aguraiuja and Wood, 2003
Asplenium haleakalense [N]							x	**I**	Kennedy *et al.*, 2010
Blechnum orientale [NZ]			**S**						Lau and Frohlich,, 2012
Ceratopteris gaudichaudii * [NZ]		x	x					**I**	Imada, 2007
Cheilanthes viridis [NZ]		x	x		**I**		x	x	Oppenheimer, 2007
Ctenitis squamigera [N]		**D**	x	x	x		x		K. Wood, 2012
Cyclosorus pendens [N]		**N**	**N**	**N**			**N**	**N**	Palmer, 2005
Davallia fejeensis [NZ]			**S**				**I**		Frohlich and Lau, 2008; D. Lorence, pers. comm.
Deparia cataracticola [N]		**N**							Kato, 2001
Dicksonia fibrosa [NZ]								**S**	Lorence and Flynn, 2006
Diplazium esculentum [NZ]		x	x	**I**	x		x	x	Oppenheimer, 2007
Elaphoglossum paleaceum [N]		x	x	x	**I**		x	x	Oppenheimer, 2010
Huperzia filiformis [N]		**I**	x	x	x		x	x	K. Wood, 2012
Lindsaea ensifolia [NZ]		x	x	**I**			x	x	Oppenheimer, 2008
Lygodium japonicum [NZ]			x					**I**	Wilson, 2003
Marsilea minuta [NZ]			**S**						Wilson, 2003
Microlepia speluncae [N]		x	x	**I**			**I**	x	Oppenheimer, 2003, 2008
Nephrolepis brownii [NZ]	**I**	x	x	x	x	x	x	x	Imada, 2007; Wood and LeGrande, 2006
Ophioglossum petiolatum [N]		x	x	**I**	x		x	x	Oppenheimer, 2010
Phymatosorus scolopendria [NZ]							**S**		Oppenheimer, 2006
Pteris lidgatei [N]			x	**D**			x		Oppenheimer, 2008
Pteris tremula [NZ]							**S**		Oppenheimer, 2007
Pteris vittata [NZ]		x	x	**I**	**I**		x	x	Oppenheimer, 2003, 2008
Pyrrosia piloselloides [NZ]			**S**						Lau and Frohlich, 2012
Sphaeropteris cooperi [NZ]		x	x		**I**		x	x	Oppenheimer, 2008
Tectaria incisa [NZ]		x	x				**I**	x	Imada, 2007

* var. vulgaris
[N] native
[NZ] naturalized

Record abbreviations: **N**=new species; **S**=new state record; **I**=new island record; **D**=rediscovery; x=previous records (Palmer, 2003); ex=possibly extinct

Island abbreviations: N=Ni'ihau; K=Kaua'i; O=O'ahu; Mo=Moloka'i; L=Lāna'i; Ma=Maui; Ka=Kaho'olawe; H=Hawai'i

federally listed or proposed for listing. As discussed later, the presumed extinct fern, *Asplenium dielmannii*, was also rediscovered on Kaua'i (Aguraiuja and Wood, 2003).

Another rediscovery on Kaua'i is that of *Ctenitis squamigera*, a species previously known from all main Hawaiian Islands with the exception of Kaho'olawe and Ni'ihau. It was last collected on Kaua'i in the late 1800's and thus presumed extinct on the island until its rediscovery in two separate locations in 2011 (K. Wood, 2012). *Pteris lidgatei* was rediscovered on the island of Moloka'i (Oppenheimer, 2007). Its last known collection on Moloka'i was in 1912, and since then had been considered extinct on the island (Oppenheimer, 2007; Palmer, 2003; W. H. Wagner *et al.*, 1999). On Moloka'i, eight individuals are known from a single population (USFWS, 2009b).

An interesting island record is that of *Psilotum nudum* (Psilotaceae). Although present on all main Hawaiian Islands including Ni'ihau and Kaho'olawe, *P. nudum* was never reported from any Northwestern Hawaiian Island (Palmer, 2003). A collection of *P. nudum* made in 1923 from Midway Atoll was discovered in 2008 in the collections of the Herbarium Pacificum (Kennedy *et al.*, 2010). Two other possible island records, based on herbarium specimens, are of *Doryopteris angelica* (Pteridaceae) and *D. takeuchii*. Yesilyurt (2005) proposed that the endangered Kaua'i endemic, *Doryopteris angelica*, has a more extensive distribution than had been previously reported (Palmer, 2003) occurring on O'ahu, Moloka'i, and Lāna'i. She also determined a range extension for the endangered (USFWS, 2012a) *Doryopteris takeuchii*, to the island of Lāna'i, previously thought to be endemic to Diamond Head Crater on O'ahu. It is imperative to note that Yesilyurt's (2005) conclusions were based on relatively few older herbarium specimens (5 specimens of *D. takeuchii* and 11 specimens of *D. angelica*), and she did not observe the type specimens for either species. For these reasons and because both of these ferns are currently federally listed endangered species it is important that new field observations be made to assess the current distribution of these two species before we accept the range extensions proposed by Yesilyurt (2005).

Newly Described Hawaiian Fern Taxa

Only one native species, *Cyclosorus pendens* (syn. *Pneumatopteris pendens*; Thelypteridaceae), has been described since Palmer's (2003) manual was published (Palmer, 2005). This species is found on Kaua'i, O'ahu, Moloka'i, Maui, and Hawai'i at elevations of 368–1220 m and is most similar morphologically to *C. sandwicensis* (Palmer, 2005). Characters that define *C. pendens* are a habitat of damp, mossy rocks often near streams and pendent, lanceolate leaves with obtuse pinnae and rachises and costae that are densely covered with hairs (Palmer, 2005).

Another species not included in Palmer (2003) is *Deparia cataracticola* (Athyriaceae), of which the author was unaware when his book was in press (D. Palmer, pers. comm.). This species is endemic to Kaua'i and is found on wet, mossy cliffs in waterfalls, a habitat similar to where the extinct *D.*

kaalaana was found (Kato, 2001). *Deparia cataracticola* is recognizable by having an ascending to erect rhizome, brown scales at the stipe base, medial sori, leaf blades that are somewhat or not tapered at the base, and by the presence of many proliferations on the lamina (Kato, 2001).

Two species of Hawaiian *Doryopteris* that were not included by Palmer (2003) have recently been recognized. Yesilyurt (2005) recognized six species of *Doryopteris* in the Hawaiian Islands, whereas Palmer (2003) recognized four species and one hybrid. The Hawaiian species of *Doryopteris* are known to have extreme inter- and intraspecific variability, leading to difficulties when circumscribing species (Palmer, 2003; Yesilyurt, 2005). For example, *Doryopteris subdecipiens* was first described by W. H. Wagner *et al.* (1999) who noted that it most likely was the result of hybridization between *D. decipiens* and *D. decora*. Palmer (2003) preferred to recognize *D. subdecipiens* as a hybrid due to its variability of characters and the continuum of intermediates between the putative parental species. The morphological study by Yesilyurt (2005) recognized this entity at the species level, although she did not rule out the possibility of it being of hybrid origin. She did not examine the type specimen and only examined nine herbarium specimens for which she noted much variation (Yesilyurt, 2005). We agree with Palmer (2003) that this species should not be recognized at the species level due to its variability and intermediate characters between the parent species. Another species, *D. tryonii*, was described in 1963 by O. Degener and I. Degener, but later synonymized under *D. decora* by Palmer (2003). Yesilyurt (2005) segregated this species based on its filiform segments, pinnate division, and crenulate indusium margins. Because the Hawaiian *Doryopteris* species exhibit high morphological variation and possibly hybridization, it is unclear whether they represent distinct species. Further studies are needed to clarify species circumscriptions.

Naturalized Hawaiian Fern Taxa

Palmer (2003) reported 33 naturalized fern and lycophyte taxa in the Hawaiian Islands. Since then, seven new state records of naturalized ferns have been reported, including taxa from previously unreported families and genera in the Hawaiian Islands (Table 3). Two previously unreported families include Davalliaceae and Dicksoniaceae, and three previously unreported genera include *Davallia* (Davalliaceae), *Dicksonia* (Dicksoniaceae), and *Pyrrosia* (Polypodiaceae).

Naturalized taxa are found on all eight main Hawaiian Islands, with O'ahu having the majority of naturalized taxa (Figure 1; Table 3). Three naturalized species, *Nephrolepis brownii* (Nephrolepidaceae), *Pityrogramma austroamericana* (Pteridaceae), and *P. calomelanos*, are found on all main Hawaiian Islands. Listed below are the seven recent new state records based on the work of Hank Oppenheimer, Ken Wilson, and the staff at the Hawai'i Biological Survey and the National Tropical Botanical Garden.

TABLE 3. Current distribution of naturalized fern and lycophyte taxa in the Hawaiian Islands.

Taxon	Island							
	N	K	O	Mo	L	Ka	Ma	H
Adiantum 'Edwinii'			x		x		x	
Adiantum hispidulum		x	x	x	x	x	x	x
Adiantum raddianum		x	x	x	x		x	x
Adiantum tenerum			x				x	
Angiopteris evecta		x	x	x	x		x	x
Azolla filiculoides		x	x	x	x		x	x
Blechnum appendiculatum		x	x	x	x		x	x
Blechnum orientale *			x					
Ceratopteris gaudichaudii var. *vulgaris*		x	x					x
Cheilanthes viridis		x	x		x		x	x
Cyclosorus dentatus		x	x	x	x		x	x
Cyclosorus parasiticus		x	x	x	x		x	x
Cyrtomium falcatum		x	x	x	x		x	x
Davallia fejeensis *			x				x	
Deparia petersenii		x	x	x	x		x	x
Dicksonia fibrosa *								x
Diplazium esculentum		x	x	x	x		x	x
Lindsaea ensifolia		x	x	x			x	x
Lygodium japonicum			x					x
Macrothelypteris torresiana		x	x				x	x
Marsilea minuta *			x					
Nephrolepis brownii	x	x	x	x	x	x	x	x
Nephrolepis falcata 'Furcans'		x	x	x	x		x	x
Nephrolepis hirsutula 'Superba'		x					x	
Phlebodium aureum		x	x	x	x		x	x
Phymatosorus grossus		x	x	x	x		x	x
Phymatosorus scolopendria *							x	
Pityrogramma austroamericana	x	x	x	x	x	x	x	x
Pityrogramma calomelanos	x	x	x	x	x	x	x	x
Platycerium bifurcatum			x				x	x
Platycerium superbum			x					
Pteris tremula *							x	
Pteris vittata		x	x	x	x		x	x
Pyrrosia piloselloides *			x					
Salvinia molesta			x					x
Selaginella kraussiana			x				x	x
Selaginella stellata								x
Selaginella umbrosa								x
Sphaeropteris cooperi		x	x		x		x	x
Tectaria incisa		x	x				x	x

* indicates new state record subsequent to Palmer (2003)
Hawaiian Island abbreviations: N=Ni'ihau; K=Kaua'i; O=O'ahu; Mo=Moloka'i; L=Lāna'i; Ma=Maui; Ka=Kaho'olawe; H=Hawai'i

Blechnum orientale (Blechnaceae) was discovered naturalized in two locations on the island of O'ahu in 2010 (Lau and Frohlich, 2012). It is native to tropical Asia, Australia, and several Pacific Islands (Chambers and Farrant, 2001; Chiou *et al.*, 1994). Diagnostic characters include rhizomes that are short,

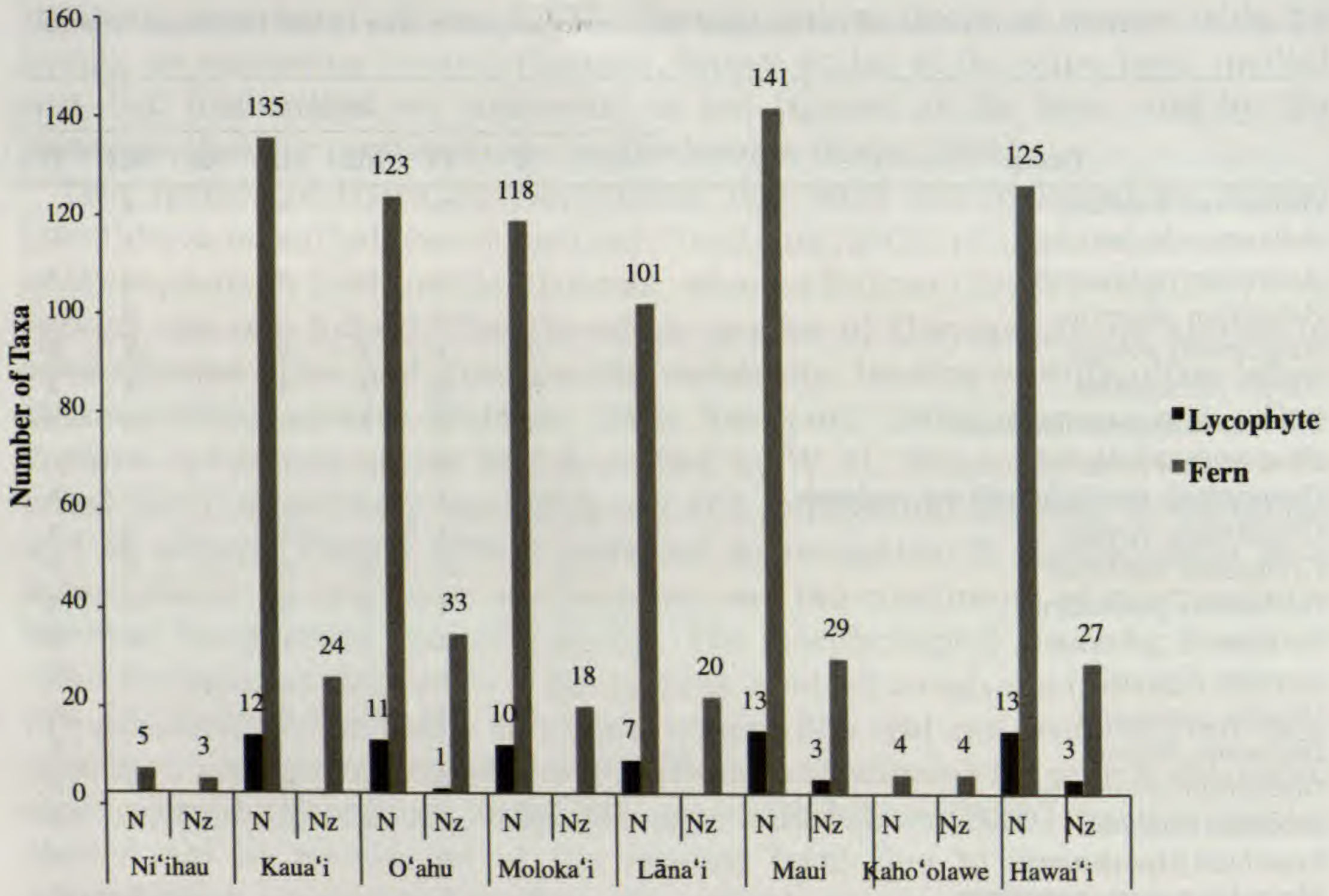

FIG. 1. Native (N) and naturalized (NZ) fern and lycophyte taxa on the eight main Hawaiian Islands. The total taxa per island includes taxa presumed extinct.

erect, and trunk-like; fronds up to 200 cm or more with blades pinnately divided; pinnae 5–30 cm long; and sori that are linear, costal, and indusiate.

Davallia fejeensis (Davalliaceae), a native to the Fiji Islands, was first reported naturalized on O'ahu in 2007 in the Nu'uanu Valley (Frohlich and Lau, 2008). The authors reported *D. fejeensis* as being widely cultivated on O'ahu and likely cultivated on other islands as well. A collection of this species (*Lorence 10375, PTBG*) was recently made near Haiku in eastern Maui (D. Lorence, pers. comm.). This species is identified by its epiphytic or sometimes terrestrial habitat, creeping rhizomes, blades that are divided 4–5 times with linear ultimate segments bearing a single vein that does not touch the margin, and sori that are solitary at segment tips (Morton, 1957; Nooteboom, 1994).

Dicksonia fibrosa (Dicksoniaceae), a New Zealand native tree fern, was reported naturalized in one locality in the South Hilo district on the island of Hawai'i (Lorence and Flynn, 2006). This species is distinguished from other Hawaiian ferns by trunks that reach 30–60 cm in diameter and up to 6 m tall, stipes with brown hairs, 20–30+ leaves per plant, blades that gradually taper towards the base, and bivalved indusia with the outer valve not differentiated from the lamina (Alan, 1982).

The first record of the aquatic fern *Marsilea minuta* (syn. *M. crenata* (Nagalingum *et al.*, 2007; Whitten *et al.*, 2012)) (Marsileaceae) is puzzling. Hillebrand (1888) reported *M. minuta* (treated as *M. crenulata*) on O'ahu,

preserved in the "Godet herbarium," although Robinson (1912a) reported that no such herbarium was known. Further investigation into the location of this specimen has been unsuccessful, although other collections from the Godet herbarium have been found at the Botanic Garden and Botanical Museum Berlin-Dahlem herbarium (B). The most recent record of *Marsilea minuta* was made in 1994 when it was found growing in taro patches at the Hawai'i Nature Center in Makiki Valley on O'ahu (Wilson, 2002, 2003). *Marsilea minuta* is native to Africa, Australia, India, and Southeast Asia (Jacona and Johnson, 2006). It is morphologically similar to the endemic *M. villosa*, which also has an aquatic habit, pinnae that are clover-like (divided into four equal pinnae), and sori enclosed in hardened sporocarps located at the bases of long stipes. This naturalized species is readily distinguished from the endemic species by its usually glabrous blades (sometimes with a few scattered hairs) with crenate pinnae margins (entire margins in aquatic forms) (Jacona and Johnson, 2006), in contrast with the glabrous blades and slightly denticulate pinnae margins of *M. villosa* (Johnson, 1986; Palmer, 2003). Furthermore, the naturalized species has roots located at nodes and internodes (Jacona and Johnson, 2006), whereas *M. villosa* has roots restricted to the nodes (Johnson, 1986).

Two populations of *Phymatosorus scolopendria* (Polypodiaceae) were reported on Maui (Oppenheimer, 2006). A collection of this species was made (*Oppenheimer & Hansen H80308,* BISH), but cannot be located for verification. *Phymatosorus scolopendria* is native to tropical Africa and Asia, Australia, and the Pacific (Brownlie, 1977). This species closely resembles and can easily be mistaken for a common naturalized fern, *P. grossus*, but is distinguished by its epiphytic habit (vs. terrestrial), smaller size, and fewer laminar lobes (Brownlie, 1977).

Pyrrosia piloselloides was found naturalized in a 2–3 acre area in the Woodlawn section of Mānoa Valley on the island of O'ahu in 2010 (Lau and Frohlich, 2012). This species is native to China, India, and Malesia (Ravensberg and Hennipman, 1986). *Pyrrosia piloselloides* is a small, epiphytic, colony-forming fern diagnosable by its sterile succulent leaves reaching 1–7 cm long, fertile linear leaves from 4–16 cm long, and linear, submarginal sori (Hovenkamp, 1986).

Pteris tremula was first reported as naturalized on Maui (Oppenheimer, 2007), when it was noted as becoming widespread throughout the central Kula areas at elevations from 914 to 1220 m. No further report has been found concerning its distributional status in the Hawaiian Islands. This species is native to Australia, New Zealand, and the Fiji Islands; it is identified by its erect leaves that attain one meter in length and blades that are 2–4 times pinnately divided with linear, toothed pinnules (Kramer and McCarthy, 1998).

Taxonomic Updates

Smith *et al.* (2006) revised the familial classification for all extant ferns based on the results of numerous molecular and morphological studies. They recognized 37 families with approximately 265 to 312 genera. A more recent

classification for extant ferns and lycophytes by Christenhusz *et al.* (2011) built upon the classification of Smith *et al.* (2006) with recent phylogenetic studies. Christenhusz *et al.* (2011) recognized 3 families with 5 lycophyte genera and 45 families with approximately 280 fern genera. Snow *et al.* (2011) addressed the need to update the taxonomy and nomenclature of the Hawaiian ferns and lycophytes. Their treatment primarily followed the classification by Smith *et al.* (2006, 2008). Rothfels *et al.* (2012b) reexamined the classification of the Eupolypods II and recognized 10 families with 36 genera. A recent classification of the Lycopodiaceae by Øllgaard (2012) accepts 9 Neotropical genera. Although these publications clarify the classification of extant ferns and lycophytes, it is important to note that many fern and lycophyte genera lack molecular studies and are in need of phylogenetic analyses to assess proposed generic delimitations (Christenhusz *et al.*, 2011; Smith *et al.*, 2006). Below, we summarize recent taxonomic and nomenclatural changes and problems that still require further study for Hawaiian fern taxa.

Ophioglossaceae.—One example of fern genera in need of further phylogenetic analysis is within the family Ophioglossaceae. The majority of treatments include *Sceptridium* within the genus *Botrychium*, and *Ophioderma* within *Ophioglossum* (Christenhusz *et al.*, 2011; Smith *et al.*, 2006, 2008). However, a molecular study by Hauk *et al.* (2003), using plastid DNA sequences from *rbcL* and *trnL-F*, showed strong support for the segregation of *Sceptridium* and *Ophioderma* as distinct genera. Snow *et al.* (2011) included *Sceptridium* within *Botrychium* and did not address the segregation of *Ophioderma*. Further phylogenetic analyses are needed to resolve these issues.

Blechnaceae.—Another example of uncertain generic boundaries is evident with the circumscription of *Blechnum*, in which Shepherd *et al.* (2007) determined *Blechnum* to be paraphyletic and including *Doodia* and *Sadleria*. In accordance with earlier work by Cranfill (2001), *Sadleria*, an endemic Hawaiian genus, was found nested within *Blechnum*. However, *S. cyatheoides* was the only species of the genus included in the study and its placement had only weak support. Christenhusz *et al.* (2011) accept the genus *Sadleria*, but state that the status is not clear due to *Blechnum* being likely paraphyletic. Rothfels *et al.* (2012b) accept the genus *Sadleria* based on the inferred phylogeny of Rothfels *et al.* (2012a). Strong support was found for *Doodia* as a monophyletic group within *Blechnum* with *B. brasiliense* Desv. as the sister species (Shepherd *et al.*, 2007). Further analyses of *Doodia* are needed before making taxonomic changes because only six of the 15–18 species of *Doodia* were sampled, including only one of the two Hawaiian endemic species (*D. kunthiana*). In addition, only one small region of plastid DNA was sequenced (*trnL-F*) and results differed depending on how the data were analyzed. Furthermore, according to the maximum likelihood tree of Shepherd *et al.* (2007), *Blechnum brasiliense* could be placed within *Doodia* to produce a monophyletic genus. Rothfels *et al.* (2012b) conserve the genus *Doodia*, whereas Christenhusz *et al.* (2011) recognize *Doodia* within the genus *Blechnum*.

ATHYRIACEAE AND CYSTOPTERIDACEAE.—Several recent classifications tentatively place the families Athyriaceae and Cystopteridaceae in Woodsiaceae, which includes four genera found in the Hawaiian Islands: *Athyrium*, *Cystopteris*, *Deparia*, and *Diplazium* (Smith *et al.* 2006, 2008; Snow *et al.*, 2011). Molecular work by Schuettpelz and Pryer (2007), using three plastid genes, revealed two members of the Woodsiaceae, *Gymnocarpium* (not present in the Hawaiian Islands) and *Cystopteris*, to be paraphyletic and sister to the eupolypods II. Based on these findings, the authors suggested that several families be recognized. Christenhusz *et al.* (2011) placed both *Cystopteris* and *Gymnocarpium*, along with two additional genera, into Cystopteridaceae. The remaining Hawaiian genera (*Athyrium*, *Deparia*, and *Diplazium*), placed within Woodsiaceae as circumscribed by Smith *et al.* (2006, 2008), are included in Athyriaceae by Christenhusz *et al.* (2011) and Rothfels *et al.* (2012b). This placement is based on results from several previously published molecular studies (Sano *et al.*, 2000; Schuettpelz and Pryer, 2007; Wang *et al.*, 2003).

NEPHROLEPIDACEAE.—The familial placement of *Nephrolepis* is unresolved. Kramer (1990) and Palmer (2003) recognized *Nephrolepis* in its own monogeneric family, Nephrolepidaceae. Smith *et al.* (2006, 2008) tentatively placed the genus within Lomariopsidaceae, which Snow *et al.* (2011) followed. Recent molecular work using plastid DNA data from three regions revealed strong support for *Cyclopeltis* and *Lomariopsis* as sister to *Nephrolepis* (Hennequin *et al.*, 2010). The authors suggested that *Nephrolepis* should be treated in its own family, Nephrolepidaceae, until further molecular analyses have been made.

POLYPODIACEAE.—Palmer (2003) recognized three Hawaiian genera of Grammitidaceae: *Adenophorus*, *Grammitis*, and *Lellingeria*. Smith *et al.* (2006, 2008) placed the family within the Polypodiaceae to resolve issues of paraphyly when Grammitidaceae is recognized (i.e., grammitid ferns form a clade that is nested within Polypodiaceae; Schneider *et al.*, 2004b). Molecular and morphological studies have shown strong evidence for *Adenophorus* as a monophyletic lineage, with *Grammitis tenella* as the sister taxon to *Adenophorus* (Ranker *et al.*, 2003, 2004). Based on these findings, Ranker (2008) transferred *Grammitis tenella* to *Adenophorus* (*A. tenellus*).

The remaining grammitid genera that include Hawaiian species, *Grammitis* and *Lellingeria*, were found to be polyphyletic (Ranker *et al.*, 2004). Strong support was found for a monophyletic clade within *Grammitis* (*Grammitis* sect. *Grammitis*) that includes the type species of the genus, *G. marginella* Sw. (Ranker *et al.*, 2004), but none of the Hawaiian species. Species within that clade share the unique character of black, sclerified leaf margins, a character not found in Hawaiian *Grammitis* (Bishop, 1977; Ranker *et al.*, 2004). In response to those findings as well as from her observations of morphology, Parris (2007) described five new genera, including reestablishing the genus *Oreogrammitis,* in which she included the three Hawaiian *Grammitis* species. Preliminary data (Ranker *et al.*, unpublished) based on plastid DNA sequences *rbcL*, *atpß*, and *trnL-trnF* found there is inadequate information to determine

the monophyly of *Oreogrammitis*. Work is in progress to further resolve the generic circumscriptions within *Grammitis* s. l. (Sundue *et al.*, unpublished). Thus, the placement of the Hawaiian members of *Grammitis* s. l. is currently unclear, although the Hawaiian species clearly do not belong to *Grammitis* s. s. due to the lack of black, sclerified leaf margins. We treat the Hawaiian species here as within *Oreogrammitis* pending further study.

Ranker *et al.* (2004) found *Lellingeria* to be divided into two non-sister clades: one including the *L. apiculata* (Kunze ex Klotzsch) A. R. Sm. & R. C. Moran and *L. myosuroides* (Sw.) A. R. Sm. & R. C. Moran groups (sister to *Melpomene* A. R. Sm. & R. C. Moran) and the other including the *L. mitchelliae* (Baker) A. R. Sm. & R. C. Moran group, now called *Leucotrichum* Labiak, (sister to *Alansmia* M. Kessler, Moguel, Sundue and Labiak) (Kessler *et al.*, 2011; Rouhan *et al.*, 2012). *Lellingeria saffordii*, the sole Hawaiian representative, is an endemic species that occurs on all major islands except Ni'ihau and Kaho'olawe. It is nested phylogenetically within the *L. myosuroides* group, which has recently been described as the new genus *Stenogrammitis* (Labiak, 2011), and thus the Hawaiian species is treated here as *S. saffordii*.

PTERIDACEAE.—*Ceratopteris* is represented in the Hawaiian Islands only by the naturalized species, *C. thalictroides*, which is found on Kaua'i, O'ahu, and Hawai'i (Imada, 2007; Palmer, 2003). Species in the genus are generally polymorphic and this is especially so with *C. thalictroides* (Masuyama and Watano, 2010). The genus has been variously recognized as including 11 species (e.g., Christensen, 1906), four species (Lloyd, 1974), with Lloyd lumping four previously described species into *C. thalictroides*, and 3 species (Tryon and Tryon, 1982). Masuyama and Watano (2010) proposed that five taxa be segregated from *C. thalictroides* based on differences in the nucleotide sequences of plastid DNA, cytological evidence, and morphological differences (Masuyama *et al.*, 2002; Masuyama and Watano, 2005; Masuyama, 2008). The *Ceratopteris* found in the Hawaiian Islands included a newly described taxon, *C. gaudichaudii* var. *vulgaris*, also known from China, Guam, Japan, Korea, Nepal, and Taiwan (Masuyama and Watano, 2010). Masuyama and Watano (2010) examined two specimens from O'ahu and determined Hawaiian members to be this new variety. Although *C. gaudichaudii* var. *vulgaris* occurs on O'ahu, it is important to note that additional *Ceratopteris* taxa may be present in the Hawaiian Islands; further study is needed.

Distribution

The majority of fern and lycophyte taxa are found on six of the eight main Hawaiian Islands (i.e., Kaua'i, O'ahu, Moloka'i, Lāna'i, Maui, and Hawai'i), with few found on Ni'ihau and Kaho'olawe (Figure 1). Two species, the endemic *Doryopteris decipiens* and the indigenous *Psilotum nudum* are distributed on all eight main Hawaiian Islands. [NB: Indigenous is defined here as taxa that are native to a particular area, but are also native elsewhere.] In total there are 140 endemic fern and lycophyte taxa, representing

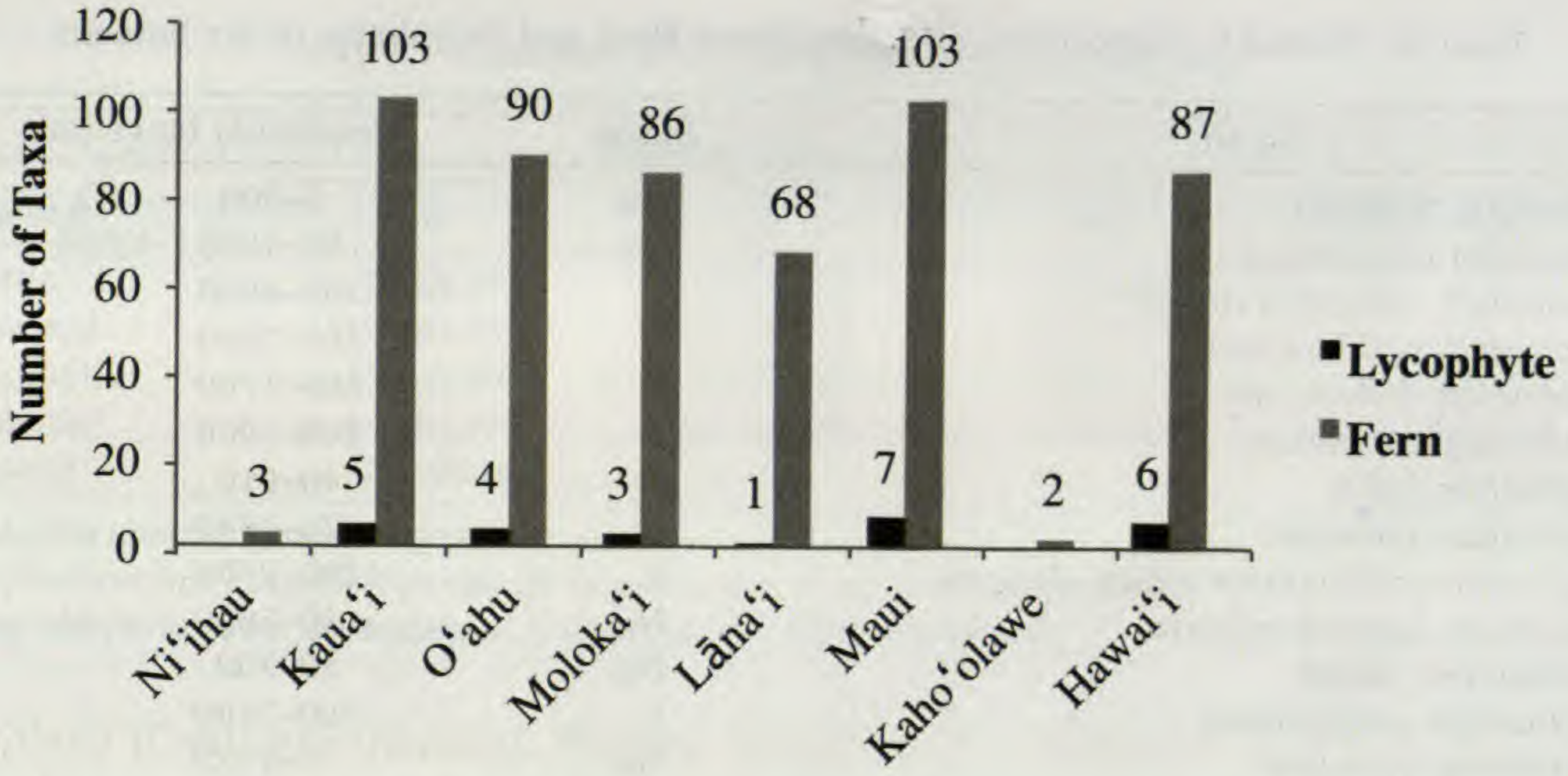

FIG. 2. Endemic fern taxa on each main Hawaiian island. The total taxa per island includes taxa presumed extinct.

approximately 77% of the 183 native taxa. This level of endemism is high compared to other oceanic islands, which usually have endemic rates of less than 50% (Moran, 2008). The greatest numbers of endemic fern and lycophyte taxa are found on Kaua'i and Maui (Figure 2). Despite the high level of island-wide endemism, Hawaiian ferns and lycophytes have relatively low numbers of single-island (28 taxa) and two-island (19 taxa) endemics (Figure 3). Only Kaua'i, O'ahu, and Maui have single-island endemics, with Kaua'i harboring the greatest number (17 taxa). The majority of taxa that are restricted to two islands are found on Maui (15 taxa) and Hawai'i (12 taxa).

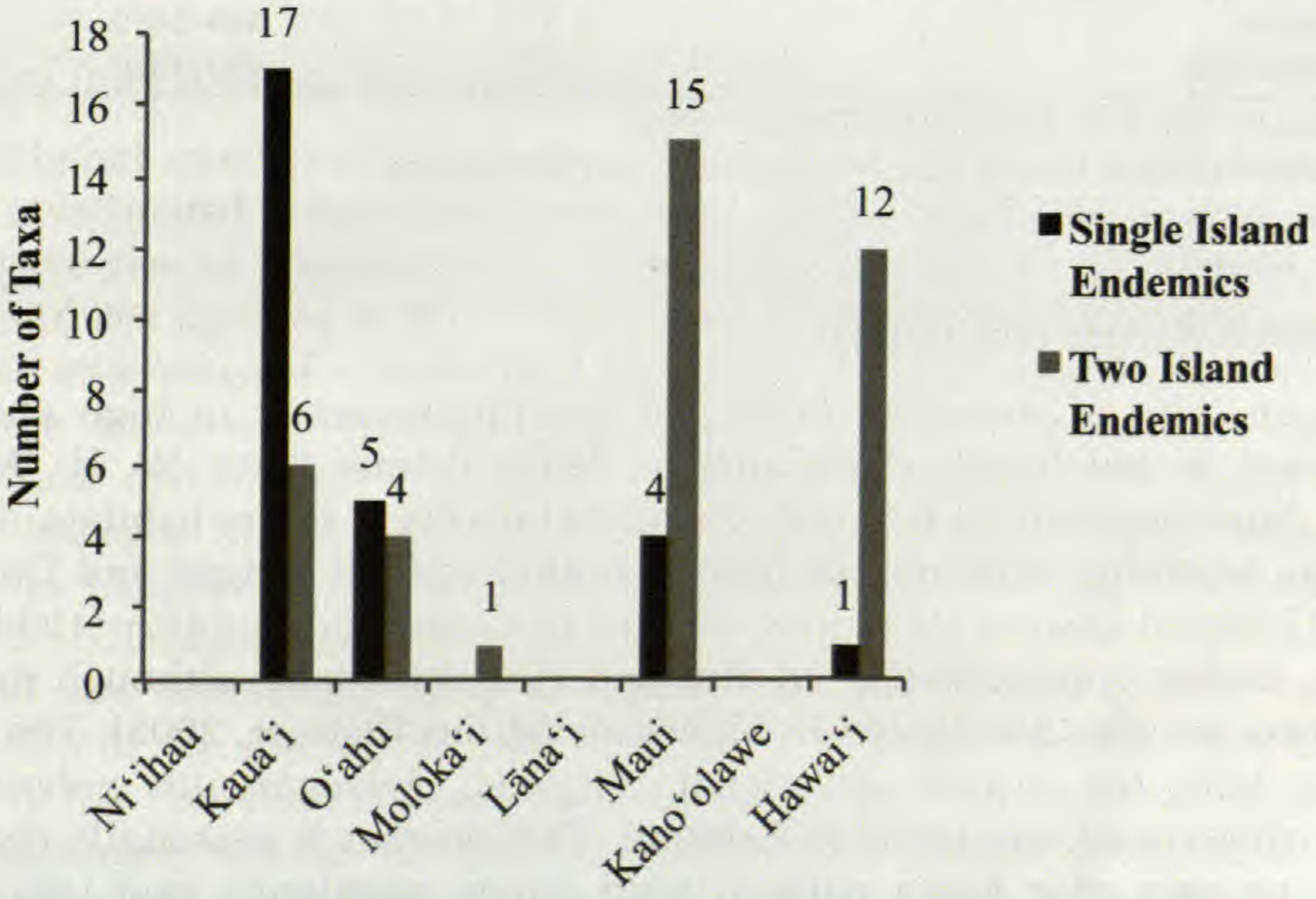

FIG. 3. Distribution of single- and two-island endemics in the eight main Hawaiian Islands. The total taxa per island includes taxa presumed extinct.

TABLE 4. Endemic, indigenous, and naturalized ferns and lycophytes of dry habitats.

Taxon	Status	Elevational range (m)
Adiantum 'Edwinii'	Nz	0–500
Adiantum hispidulum	Nz	90–1250 (–1825)
Asplenium adiantum-nigrum	I	350–4000
Asplenium aethiopicum *	I	305–1950
Asplenium dielerectum	E	500–1700
Asplenium caudatum	I	200–1000
Asplenium nidus	I	40–610
Asplenium normale	I	375–1680
Asplenium trichomanes subsp. *densum*	E	1200–2700
Blechnum appendiculatum	Nz	30–560
Cheilanthes viridis	Nz	10–700
Cyrtomium caryotideum	I	300–2100
Cyrtomium falcatum	Nz	0–1525
Doodia kunthiana	E	90–1220
Doryopteris decipiens	E	30–915
Doryopteris decora	E	120–700
Doryopteris takeuchii	E	100–120
Dryopteris fuscoatra var. *fuscoatra*	E	500–2100
Lepisorus thunbergianus	I	10–2100
Lycopodium venustulum var. *verticale*	E	1220–2440
Microlepia speluncae	I	480–1280
Microlepia strigosa var. *mauiensis*	E	0–1770
Microlepia strigosa var. *strigosa*	I	425–1830
Nephrolepis brownii	Nz	low elevations
Pellaea ternifolia	I	600–3500
Phlebodium aureum	Nz	0–700
Pityrogramma austroamericana	Nz	45–1525
Pityrogramma calomelanos	Nz	0–1000
Pteris cretica	I	340–2450
Pteris irregularis	E	450–1920

*occasionally found in dry habitats (Palmer, 2003)
Status abbreviations: E=endemic; I=indigenous; Nz=naturalized

Elevational Ranges and Habitat

The majority of Hawaiian ferns and lycophytes occur in high elevation mesic-wet to wet forests (MacCaughey, 1918; Palmer 2003; W. H. Wagner, 1995). Approximately 31 fern and lycophyte taxa occur in dry habitats, defined as areas receiving 1200 mm or less of annual rainfall (Gagné and Cuddihy, 1999) (Table 4). Coastal elevations, defined by Gagné and Cuddihy (1999) as 0–300 m, harbor approximately 48 fern and lycophyte taxa, although many of these taxa are also distributed in higher elevations (Palmer, 2003). The native species with the lowest elevational range is *Ophioglossum polyphyllum* (Ophioglossaceae), occurring at 2–160 m. This species is seasonally common, appearing only after heavy rains in sand dunes, grasslands, and lava cobble (Palmer, 2003). Native species that occur at some of the highest elevations (above 3000 m) include: *Asplenium adiantum-nigrum* (Aspleniaceae), *Cystopteris*

TABLE 5. Summary of fern and lycophyte taxa habitats[1].

Habitat	Number of taxa (%)			Total taxa
	E	I	Nz	
Aquatic	2 (28.57)	1 (14.29)	4 (57.14)	**7**
Terrestrial	117 (62.23)	39 (20.75)	32 (17.02)	**188**
Lithophytic	23 (63.89)	9 (25.00)	4 (11.11)	**36**
Epiphytic*	44 (66.67)	15 (22.73)	7 (10.61)	**66**
Epigeous	3 (100.00)	0	0	**3**

[1] includes species presumed extinct

* *Vandenboschia davallioides* can be hemi-epiphytic as well as epiphytic

Status abbreviations: E=endemic; I=indigenous; Nz=naturalized

douglasii (Cystopteridaceae), *Pellaea ternifolia* (Pteridaceae), and *Polystichum haleakalense* (Dryopteridaceae).

The majority of Hawaiian ferns are terrestrial, although many are epiphytic and occur mostly in high elevation, wet forests (Table 5). Several species are restricted to aquatic habitats, including the endangered endemic water clover fern, *Marsilea villosa* and the rare lycophyte, *Isoëtes hawaiiensis* (Isoëtaceae). Other interesting habitats include those that are epigeous (growing above, but near the surface of the ground in moss mats at the base of trees) and lithophytic/epipetric (growing on rocks). Only three taxa have an epigeous habit, and all are within the endemic genus *Adenophorus*: *A. epigaeus*, *A. tamariscinus* var. *tamariscinus*, and *A. tripinnatifidus*. Several examples of lithophytic species include *Asplenium dielerectum*, *Cyclosorus boydiae*, *C. pendens*, and *Doryopteris takeuchii*.

Origins of the Hawaiian fern and lycophyte flora

The original number of colonizing species of angiosperms to the Hawaiian Islands is estimated to have been a minimum of 291 species (Sakai *et al.*, 1995), which gave rise to approximately 1032 native species (W. L. Wagner, pers. comm. and see updates at W. L. Wagner *et al.*, 2005–) in the flowering plant flora (i.e., an average of ~3.6 derived species per colonizing ancestor). We used data from molecular phylogenetic studies and/or information on the geographical distribution of species and genera to estimate the origins of 78 taxa or lineages (33 endemic and 45 indigenous) of Hawaiian ferns and lycophytes (Appendix 2). Molecular phylogenetic data were available for 22 taxa and/or lineages (see references cited in Appendix 2). We inferred the origin of the remaining 56 taxa and/or lineages based on the current distribution of the species (i.e., for indigenous species of limited distribution outside of the Hawaiian Islands) or of the genus (i.e., for Hawaiian endemics with congeneric species of limited distribution outside of the Hawaiian Islands). The 78 taxa or lineages comprise 103 taxa in total, thus there are an average of ~1.3 extant taxa per inferred colonizing species (Table 6 and Appendix 2). We estimate that the majority of Hawaiian fern and lycophyte

TABLE 6. Estimated source regions for the origins of Hawaiian fern and lycophyte lineages[1].

Distribution of lineage sister to Hawaiian lineage	E (%)	I (%)	N (%)
Asia/Paleotropics	21 (64)	25 (56)	46 (59)
Neotropics/Americas	6 (18)	5 (11)	11 (14)
Boreal	2 (6)	1 (2)	3 (4)
Pantropical	2 (6)	10 (22)	12 (15)
Cosmopolitan	1 (3)	2 (4)	3 (4)
South Pacific	0 (0)	2 (4)	2 (3)
Austral	1 (3)	0 (0)	1 (1)
Total known/estimated	33	45	78
Total unknown	60	0	60

[1] includes species presumed extinct

Status abbreviations: E=endemic; I=indigenous; N=native (endemic + indigenous lineages)

lineages (59%) are of Asian/Paleotropical origin (Table 6); that broad area is the most distant from the Hawaiian Islands compared to other possible source regions. Other hypothesized source regions include Pantropical (15%, but directionality unknown), Americas/Neotropics (14%), Cosmopolitan (4%, but directionality unknown), Boreal (4%), South Pacific (3%), and Austral (1%) (Table 6). Geiger *et al.* (2007 and references therein) identified four climate- or weather-based phenomena that might allow spores to be carried great distances to the Hawaiian Islands: 1) the northern subtropical jet stream from southeast Asia/Malaysia, 2) northern trade winds from America, 3) tropical storms from Mexico and Central America, and 4) a Hadley Cell shift from the tropical convergence zone that would allow for the Marquesas Islands to be a source area (Geiger *et al.*, 2007; Wright *et al.*, 2001).

Many Hawaiian angiosperm genera are believed to be derived from single colonization events with subsequent diversifications, including the Hawaiian silverswords (Asteraceae), *Bidens* (Asteraceae), and *Schiedea* (Caryophyllaceae) (Baldwin and Wagner, 2010). This contrasts with Hawaiian fern genera, which are often the result of multiple colonization events. For example, several biogeographical studies focused on determining the origins of Hawaiian Dryopteridaceae. Geiger and Ranker (2005) used chloroplast DNA (cpDNA) sequences from *rbcL* and *trnL-F* to explore the origins of Hawaiian *Dryopteris* species. They found Hawaiian *Dryopteris* to be the result of multiple colonizations and hypothesized five separate colonization events, most of these likely of Southeast Asian origin. In another study of Dryopteridaceae, Driscoll and Barrington (2007) used the same cpDNA sequences as Geiger and Ranker (2005) to explore the origins of Hawaiian *Polystichum* species. They determined it to be polyphyletic as well, with *P. hillebrandii* being of separate continental eastern Asian origins from the remaining two species, *P. haleakalense* and *P. bonseyi*.

Considerable work also has been conducted to determine the biogeographical origins of the Hawaiian grammitid ferns (*Adenophorus*, *Oreogrammitis* (formerly *Grammitis*, in part), and *Stenogrammitis* (syn. *Lellingeria*)). Ranker

et al. (2004) used cpDNA sequences from *rbcL* and *atpß* to explore the phylogeny of the grammitid ferns. Their results suggested three colonization events to the Hawaiian Islands. Species of the endemic Hawaiian genus *Adenophorus* (10 spp.) were hypothesized to have originated from a single Neotropical ancestor and further analyses from Geiger *et al.* (2007) provided additional support for this hypothesis. The origins of Hawaiian *Oreogrammitis* (3 spp.) are unclear. Ranker *et al.* (2004) postulated a southwest Pacific origin based on the sister relationship of *O. knutsfordiana* (Baker) Parris to the Hawaiian *Oreogrammitis* clade. Geiger *et al.* (2007) further supported this hypothesis, but the exact origins of the Hawaiian clade are unknown due to the widespread distribution of *O. knutsfordiana*. The third genus of Hawaiian grammitids, *Stenogrammitis* (1 sp.), is believed to be of Neotropical origin. Geiger *et al.* (2007) determined the Hawaiian species *S. saffordii* to be sister to *S. subcoriacea* (Copel.) Labiak from the Marquesas and Society Islands. Because this clade is sister to the Neotropical species *S. limula*, they hypothesized a Neotropical origin for the Hawaiian *Stenogrammitis*. A recent study by Labiak *et al.* (2010) suggests a Neotropical origin, but provided support for *S. saffordii* as being sister to the Mexican species *S. hellwigii* (Mickel & Beitel) Labiak.

Multiple colonizations like those for the Dryopteridaceae also have been postulated for Hawaiian *Doryopteris* (Yesilyurt, 2005) and the indigenous *Asplenium adiantum-nigrum* (Ranker *et al.*, 1994; Shivas, 1969). According to Yesilyurt (2005) there are molecular data (Yesilyurt *et al.* in prep) that suggest *D. decora* and *D. decipiens* are the result of separate introductions to the Hawaiian Islands. Another Hawaiian fern, the aquatic *Marsilea villosa*, has been hypothesized to be of North American ancestry. Nagalingum *et al.* (2007) used six plastid regions to determine strong support for *M. villosa* as being sister to *M. oligospora* Goodd. and *M. vestita* Hook. & Grev., both North American temperate species. Molecular work by Whitten *et al.* (2012) found additional support for a North American origin. Shivas (1969) hypothesized *Asplenium adiantum-nigrum* to be of hybrid origin from two diploid European taxa based on cytological data. Ranker et al. (1994) used isozyme data to infer multiple colonization events of this species to the Hawaiian Islands.

Conservation of Hawaiian Ferns

Conservation of the Hawaiian flora and preservation of the native forests is of most immediate concern in the Hawaiian Islands. Extinction rates within the United States are highest in the Hawaiian Islands (Gagné, 1988). Despite this high level of extinction, only three historically documented fern species and one lycophyte species are presumed to be extinct in the Hawaiian Islands (Table 7). The primary threat to the decline of Hawaiian ferns and lycophytes is habitat destruction by humans and feral animals (Given, 1993; W. H. Wagner, 1995). Habitat destruction of the Hawaiian Islands began with the Polynesian settlers, but increased rapidly after Western contact (Gagné, 1988). The introduction of feral animals (e.g., pigs, sheep, cattle, deer, goats) and

TABLE 7. Federal and state status of Hawaiian ferns and lycophytes.

Taxon	Date Federally Listed	Status				
		X	E	C	SC	SGCN
Adenophorus periens **	11/10/1994		x			x
Asplenium dielerectum **	11/10/1994		x			x
Asplenium dielfalcatum	10/29/1991		x			x
Asplenium dielmannii **	05/13/2010		x		x	x
Asplenium dielpallidum **	02/25/1994		x			x
Asplenium leucostegioides	-	x			x	
Asplenium peruvianum var. *insulare* **	09/26/1994		x			x
Asplenium schizophyllum	-				x	
Asplenium unisorum	06/27/1994		x			x
Athyrium microphyllum *	-					x
Cibotium chamissoi *	-					x
Cibotium glaucum *	-					x
Cibotium spp.	-					x
Ctenitis squamigera	09/26/1994		x			x
Cyclosorus boydiae	-			x		
Cyclosorus interruptus *	-					x
Cyclosorus wailele	-				x	
Cystopteris douglasii	-				x	
Deparia kaalaana	-	x			x	
Dicranopteris linearis *	-					x
Diplazium molokaiense **	09/26/1994		x			x
Diplazium sandwichianum *	-					x
Doryopteris angelica **	05/13/2010		x			x
Doryopteris takeuchii	09/18/2012		x			
Dryopteris crinalis var. *podosora* **	05/13/2010		x		x	x
Dryopteris tetrapinnata	-				x	x
Dryopteris spp.	-					x
Elaphoglossum spp.	-					
Huperzia haleakalae ***	-	x				
Huperzia mannii	05/15/1992		x			x
Huperzia nutans	03/28/1994		x			x
Huperzia stemmermanniae **	-			x		x
Isoetes hawaiiensis	-				x	
Marsilea villosa *	06/22/1992		x			x
Microlepia strigosa var. *mauiensis*	-			x		x
Pteris lidgatei **	09/26/1994		x			x
Sadleria cyatheoides *	-					x
Sceptridium subbifoliatum	-	x			x	

* important component of native habitat (Mitchell *et al.*, 2005)
** fewer than 50 individuals in the wild (PEP, 2011)
*** extant elsewhere, extinct only in the Hawaiian Islands (W. L. Wagner *et al.*, 2005–)
Status designation: X=extinct (Wagner *et al.*, 2005–); E=endangered (USFWS, 2012a; 2012b); C=candidate (USFWS, 2012b); SC=species of concern (DOFAW, 2001); SGCN =species of greatest conservation need (Mitchell *et al.*, 2005)

non-native plant species has further contributed to the habitat loss and population decline in Hawaiian ferns (W. H. Wagner, 1995).

Approximately 1366 non-native vascular plant species have been introduced to the Hawaiian Islands and have since become naturalized (C. Imada, pers. comm. and W. L. Wagner *et al.*, 2012). These plants were introduced to the Hawaiian Islands primarily for their use in the horticultural trade, although some introductions were accidental. Non-native, alien taxa have the potential to become naturalized and possibly invasive by competing with the native flora. Over 260 species of cultivated alien ferns are estimated to have been introduced to the Hawaiian Islands mainly for the horticultural trade and use in botanical gardens (Robinson *et al.*, 2010; Wilson, 1996). The most recent publications (Palmer, 2003; Wilson, 2002) report 33 naturalized fern and lycophyte taxa, all of which were likely introduced to the islands after 1880 (Wilson, 2002). As stated above, since Palmer (2003) was published, seven new fern taxa have been reported as naturalized in the state (Table 3).

Single, invasive fern species have caused major problems in many parts of the world (Robinson *et al.*, 2010). By contrast, at least 18 species in the Hawaiian Islands have become invasive based on their abundance and widespread distribution throughout the islands (Palmer, 2003). Invasive species often are highly detrimental to native ecosystems due to their ability to outcompete and displace the native flora. For example, the introduced tree fern *Sphaeropteris cooperi* (Cyatheaceae) grows faster and produces more leaves than the native tree ferns, *Cibotium* spp. (Cibotiaceae) (Durand and Goldstein, 2001). Another example is of the common, naturalized fern *Blechnum appendiculatum*, reported as one of the two plants completely overgrowing a population of the endangered fern *Asplenium dielpallidum* (Aguraiuja *et al.*, 2004).

Several naturalized fern species hybridize with native Hawaiian species. For example, the naturalized fern *Cyclosorus dentatus* has crossed with the endemic species *Cyclosorus cyatheoides* (*Cyclosorus* ×*palmeri* (W. H. Wagner) W. H. Wagner) on Kaua'i and O'ahu (W. H. Wagner *et al.*, 1999). Another example is of an intergeneric hybrid (×*Lindsaeosoria flynnii* W. H. Wagner), the naturalized *Lindsaea ensifolia* (Lindsaeaceae) crossing with the indigenous *Sphenomeris chinensis* (Lindsaeaceae) (W. H. Wagner, 1993). The effects of naturalized species crossing with native species are not entirely known, although these hybrids have the potential to threaten native biodiversity due to the effects of outbreeding depression and introgression (Bleeker *et al.*, 2007).

Many Hawaiian endemic ferns are difficult to cultivate, likely because of their requirement of highly specialized habitats (W. H. Wagner, 1995). Examples of this are ferns with green spores (grammitid ferns and Hymenophyllaceae) that germinate immediately, given the proper environmental factors (Mehltreter, 2010). However, if proper environmental conditions are not met, the chlorophyll in the spores can quickly break down, and viability of the spores may be reduced to approximately seven weeks (Mehltreter, 2010). Propagation of these ferns can be difficult and expensive due to the need for specific environmental conditions that are not always

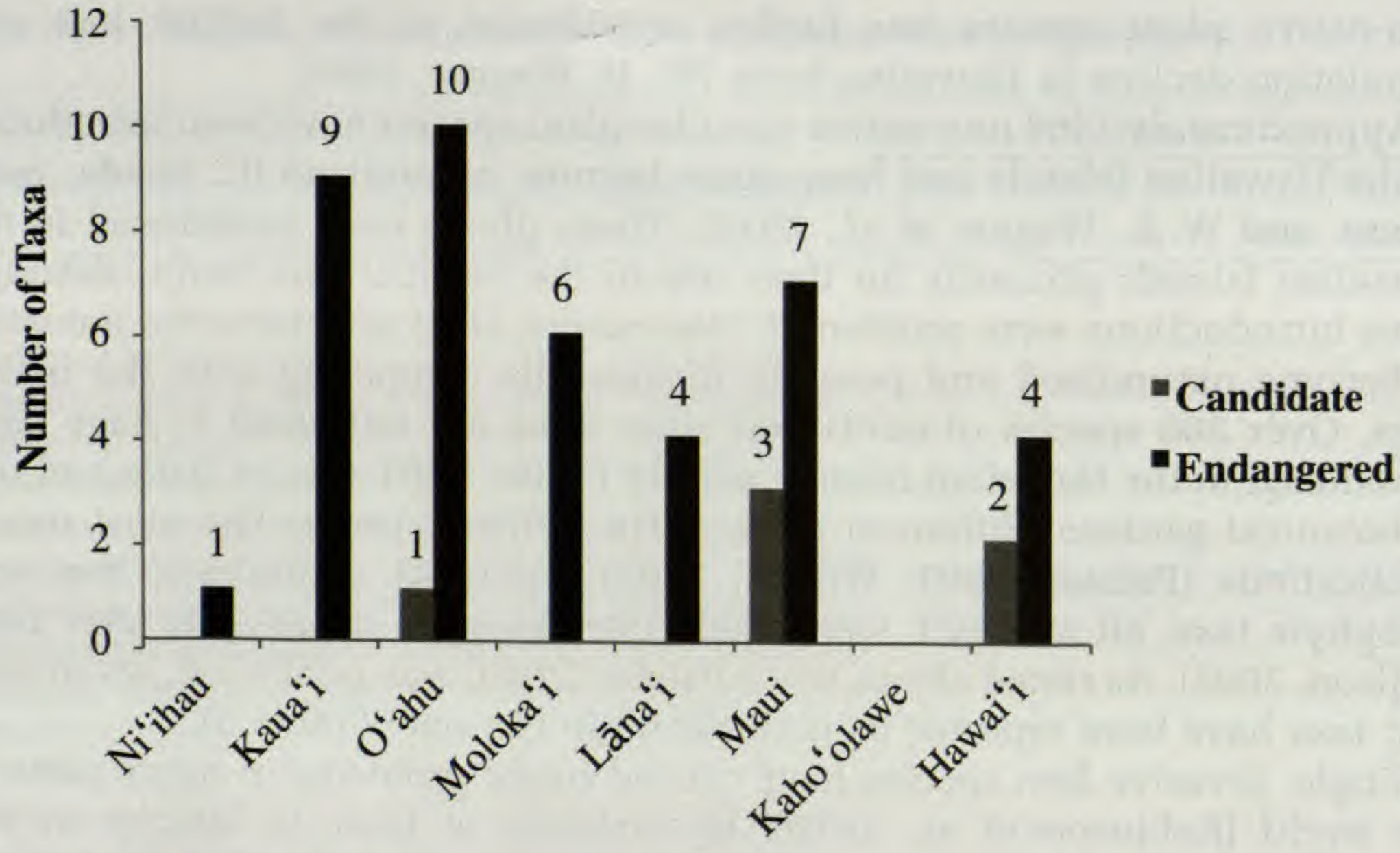

FIG. 4. Federally listed endangered, proposed, and candidate taxa on each of the main Hawaiian Islands. The total taxa per island includes taxa presumed extinct.

achievable in the traditional greenhouse settings (W. H. Wagner, 1995). It is therefore critical that these species are identified so accurate environmental assessments are documented and land be preserved that can host these endemic species.

Endangered Hawaiian Ferns

As of 2012, the U. S. Fish and Wildlife Service had listed 339 Hawaiian plant taxa as either threatened (9 taxa) or endangered (330 taxa). Of those, 5% are ferns (14 taxa) and lycophytes (2 taxa) and all are listed as endangered (Table 7). *Doryopteris takeuchii*, a fern endemic to Diamond Head Crater on O'ahu, was recently listed as endangered (USFWS, 2012a). In addition, three taxa are candidate species: *Cyclosorus boydiae*, *Microlepia strigosa* var. *mauiensis*, and *Huperzia stemmermanniae* (Lycopodiaceae). The greatest number of federally listed (including endangered and candidate taxa) ferns and lycophytes occur on O'ahu, although Kaua'i and Maui have nearly the same number (Figure 4). One taxon, *Dryopteris glabra* var. *pusilla*, was formerly listed as a candidate species, but has been removed due to the likelihood that the species has a greater distribution than previously thought; additional surveys are needed (USFWS, 2004).

The Hawai'i Department of Fish and Wildlife (DOFAW, 2001) identified nine fern taxa and one lycophyte as "species of concern" and in greatest need of conservation efforts (Table 7). W. L. Wagner *et al.* (2005–) consider three of these to be extinct (i.e., *Asplenium leucostegioides*, *Deparia kaalaana*, and *Sceptridium subbifoliatum* (Ophioglossaceae)) and two are currently federally listed as endangered (*Asplenium dielmannii* and *Dryopteris crinalis* var. *podosora*).

TABLE 8. Reported individuals of federally listed endangered Hawaiian ferns and lycophytes in wild populations.

Taxon	Reported individuals	Reference
Adenophorus periens	15–123	USFWS, 2010a
Asplenium dielerectum	<100	USFWS, 2009c
Asplenium dielfalcatum	thousands	USFWS, 2011a
Asplenium dielmannii	~60	K. Wood, pers. comm.
Asplenium dielpallidum	12	USFWS, 2008
Asplenium peruvianum var. *insulare*	278	USFWS, 1998
Asplenium unisorum	1148	USFWS, 2011b
Ctenitis squamigera	234–242	USFWS, 2009d
Diplazium molokaiense	~65	USFWS, 2010b
Doryopteris angelica	29–54	USFWS, 2011c
Doryopteris takeuchii	50–100	USFWS, 2012a
Dryopteris crinalis var. *podosora*	32–47	USFWS, 2011d
Huperzia mannii	61–71	USFWS, 2011e
Huperzia nutans	11	USFWS, 2009e
Marsilea villosa	thousands	USFWS, 2011f
Pteris lidgatei	51	USFWS, 2009b

In 2005, DOFAW and the Division of Aquatic Resources (DAR) within the Department of Land and Natural Resources (DLNR) developed a Comprehensive Wildlife Conservation Strategy (CWCS) to review the status of the native terrestrial and aquatic species in the Hawaiian Islands (Mitchell *et al.*, 2005). The CWCS identifies the Hawaiian species of greatest conservation need (SGCN) and provides strategic plans for their conservation. The criteria CWCS used to determine a SGCN need may be found at: http://www.state.hi.us/dlnr/dofaw/cwcs/process_strategy.htm. The CWCS includes 22 ferns and 3 lycophytes as species of greatest conservation need (Table 7). Eight of these species are listed as SGCN because they play an important role in their native habitats as either a dominant member of the native plant community or by hosting or providing habitat for other native species (Mitchell *et al.*, 2005). Along with these species, three genera (*Cibotium*, *Dryopteris*, and *Elaphoglossum*) also have this designation (Mitchell *et al.*, 2005).

The Plant Extinction Prevention (PEP) program of Hawai'i reported nine fern taxa and one lycophyte with less than 50 individuals remaining in wild populations: *Adenophorus periens*, *Asplenium dielerectum*, *A. dielmannii*, *A. dielpallidum*, *Asplenium peruvianum* var. *insulare*, *Diplazium molokaiense*, *Doryopteris angelica*, *Dryopteris crinalis* var. *podosora*, *Huperzia stemmermanniae*, and *Pteris lidgatei* (PEP, 2011). Approximate numbers of currently known individuals of endangered Hawaiian ferns have been compiled from reports of the USFWS and additional resources (Table 8).

Extinct Hawaiian Ferns

W. L. Wagner *et al.* (2005–) reported three ferns and one lycophyte from the Hawaiian Islands as extinct (Table 7). A single plant of one previously

believed extinct species (HBS, 1999), *Asplenium dielmannii* (syn. *Diellia mannii*), was rediscovered on Kaua'i in 2003 (Aguraiuja and Wood, 2003). Prior to this discovery, the last known collections were made in western Kaua'i sometime between 1871 and 1886 (Aguraiuja and Wood, 2003). There have been further discoveries of this species on the island of Kaua'i. Another population of approximately 60 individuals was discovered in 2010 and a fence was built around the population for protection (K. Wood, pers. comm.). No additional rediscoveries of presumed extinct ferns have been reported, but it is difficult, if not impossible, to know with certainty when a species is definitely extinct. As Palmer (2003) suggested, the presumed extinct fern, *Sceptridium subbifoliatum*, may be overlooked in the field due to the similarity of its young leaves to those of *Marattia douglassii* (Marattiaceae).

Hawaiian ferns and lycophytes are important components in native Hawaiian ecosystems and it is therefore necessary that these plants be bettered understood and protected. We hope this publication along with the interactive key will evoke interest and further educate those interested in Hawaiian ferns and lycophytes.

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APPENDIX 1. Checklist for the native and naturalized ferns and lycophytes of the Hawaiian Islands. The checklist follows the linear sequence for extant ferns and lycophytes by Christenhusz *et al.* (2011) with several modifications (Rothfels *et al.*, 2012b).

[E] endemic; [I] indigenous; [NZ] naturalized

Hawaiian Island abbreviations: N=Ni'ihau; K=Kaua'i; O=O'ahu; Mo=Moloka'i; L=Lāna'i; Ma=Maui; Ka=Kaho'olawe; H=Hawai'i

(ex)=extinct

* synonyms listed are those that represent updates subsequent to Palmer (2003)

LYCOPHYTES

1. **Lycopodiaceae** P. Beauv. ex Mirb.

Huperzia Bernh.

[E] ***Huperzia erosa*** Beitel & W. H. Wagner

Distribution: K/O/Mo/L/Ma/H

[I] ***Huperzia erubescens*** (Brack.) Holub

Distribution: K/O/Mo/Ma/H

[I] ***Huperzia filiformis*** (Sw.) Holub

Distribution: K/O/Mo/L/Ma/H

[I] ***Huperzia haleakalae*** (Brack.) Holub

Distribution: Ma (ex)

[E] ***Huperzia mannii*** (Hillebr.) Kartesz & Gandhi

Distribution: K (ex)/Ma/H

[E] ***Huperzia nutans*** (Brack.) Rothm.

Distribution: K (ex)/O

[I] ***Huperzia phyllantha*** (Hook. & Arn.) Holub

Distribution: K/O/Mo/L/Ma/H

[I] ***Huperzia serrata*** (Thunb. ex Murray) Trevis.

Distribution: K/O/Mo/L/H

[E] ***Huperzia stemmermanniae*** (A. C. Medeiros, W. H. Wagner & Hobdy) Kartesz

Distribution: Ma/H

[E] ***Huperzia subintegra*** (Hillebr.) Beitel & W. H. Wagner

Distribution: K/O/Mo/Ma

Lycopodiella Holub

[I] ***Lycopodiella cernua*** (L.) Pic. Serm.

Distribution: K/O/Mo/L/Ma/H

Lycopodium L.

[I]***Lycopodium venustulum*** Gaudich. var. ***venustulum***

Distribution: K/O/Mo/L/Ma/H

[E] ***Lycopodium venustulum*** Gaudich. var. ***verticale*** W. H. Wagner

Distribution: H

2. **Isoëtaceae** Reichenb.

Isoëtes L.

[E] ***Isoëtes hawaiiensis*** W. C. Taylor & W. H. Wagner

Distribution: Ma/H

3. **Selaginellaceae** Willk.

Selaginella P. Beauv.

[I]***Selaginella arbuscula*** (Kaulf.) Spring

Distribution: K/O/Mo/L/Ma/H

note: Selaginella arbuscula was previously considered an endemic species. This species has a greater distribution, occurring in the Society Islands, Ualan, Santa Cruz Island (Vanikoro), and the Marquesas Islands (Hassler and Swale, 2003).

[E] ***Selaginella deflexa*** Brack.

Distribution: K/O/Mo/Ma/H

[NZ] ***Selaginella kraussiana*** (Kunze) A. Braun

Distribution: O/Ma/H

[NZ] ***Selaginella stellata*** Spring

Distribution: H

[NZ] ***Selaginella umbrosa*** Lem. ex Hieron.

Distribution: H

FERNS

1. **Ophioglossaceae** Martinov

Sceptridium Lyon

[E] ***Sceptridium subbifoliatum*** (Brack.) Lyon

Distribution: K (ex)/O (ex)/Mo (ex)/L (ex)/Ma (ex)/H (ex)

Ophioderma (Blume) Endl.

Ophioderma pendulum (L.) C. Presl

[I] subsp. ***falcatum*** (C. Presl) R. T. Clausen

Distribution: K/O/Mo/L/Ma/H

[I] subsp. ***pendulum***

Distribution: O/Mo/Ma/H

Ophioglossum L.

[I]***Ophioglossum nudicaule*** L. f.

Distribution: K/O/H

[I]***Ophioglossum petiolatum*** Hook.

Distribution: K/O/Mo/L/Ma/H

[I]***Ophioglossum polyphyllum*** A. Braun

Distribution: K/O/Mo/L (ex)/Ka/Ma/H

2. **Psilotaceae** J.W.Griff. & Henfr.

Psilotum Sw.

[I]***Psilotum complanatum*** Sw.

Distribution: K/O/Mo/L/Ma/H

[I]***Psilotum nudum*** (L.) P. Beauv.

Distribution: N/K/O/Mo/L/Ka/Ma/H

3. **Marattiaceae** Kaulf.

Angiopteris Hoffm.

[NZ] ***Angiopteris evecta*** (G. Forst.) Hoffm.

Distribution: K/O/Mo/L/Ma/H

Marattia Sw.

[E] ***Marattia douglasii*** (C. Presl) Baker

Distribution: K/O/Mo/L/Ma/H

4. **Hymenophyllaceae** Mart.

Callistopteris Copel.

[E] ***Callistopteris baldwinii*** (D. C. Eaton) Copel.

Distribution: K/O/Mo/L/Ma/H

Crepidomanes C. Presl

[E] ***Crepidomanes draytonianum*** (Brack.) Ebihara & K. Iwats.

Syn. *Vandenboschia draytoniana* (Brack.) Copel.

Distribution: K/O/Mo/L/Ma/H

[I]***Crepidomanes minutum*** (Blume) K. Iwats.

Syn. *Gonocormus minutus* (Blume) Bosch

Distribution: K/O/Mo/L/Ma/H

[I] ***Crepidomanes proliferum*** (Blume) Bostock

Syn. *Gonocormus prolifer* (Blume) Prantl

Distribution: Ma/H

Hymenophyllum Sm.

[E] ***Hymenophyllum lanceolatum*** Hook. & Arn.

Syn. *Sphaerocionium lanceolatum* (Hook. & Arn.) Copel.

Distribution: K/O/Mo/L/Ma/H

[E] ***Hymenophyllum obtusum*** Hook. & Arn.

Syn. *Sphaerocionium obtusum* (Hook. & Arn.) Copel.

Distribution: O/Mo/L/Ma/H

[E] ***Hymenophyllum recurvum*** Gaudich.

Syn. *Mecodium recurvum* (Gaudich.) Copel.

Distribution: K/O/Mo/L/Ma/H

Vandenboschia Copel.

[E] ***Vandenboschia cyrtotheca*** (Hillebr.) Copel.

Distribution: K/O/Mo/L/Ma/H

[E] ***Vandenboschia davallioides*** (Gaudich.) Copel.

Distribution: K/O/Mo/L/Ma/H

[E] ***Vandenboschia tubiflora*** F. S. Wagner

Distribution: K

5. **Gleicheniaceae** C. Presl

Dicranopteris Bernh.

Dicranopteris linearis (Burm. f.) Underw.

Distribution: K/O/Mo/L/Ma/H

Diplopterygium (Diels) Nakai

[E] ***Diplopterygium pinnatum*** (Kunze) Nakai

Distribution: K/O/Mo/L/Ma/H

Sticherus C. Presl

[E] ***Sticherus owhyhensis*** (Hook.) Ching

Distribution: K/O/Mo/L/Ma/H

6. **Lygodiaceae** M. Roem.

Lygodium Sw.

[NZ] ***Lygodium japonicum*** (Thunb.) Sw.

Distribution: O/H

7. **Schizaeaceae** Kaulf.

Schizaea Sm.

[E] ***Schizaea robusta*** Baker

Distribution: K/O/Mo/L/Ma/H

8. **Marsileaceae** Mirb.

Marsilea L.

[NZ] ***Marsilea minuta*** L.

Syn. *Marsilea crenata* C. Presl

Distribution: O

[E] ***Marsilea villosa*** Kaulf.

Distribution: N/O/Mo

9. **Salviniaceae** Martinov

Salvinia Seg.

[NZ] ***Salvinia molesta*** D. S. Mitch.

Distribution: O/H

Azolla Lam.

[NZ] ***Azolla filiculoides*** Lam.

Distribution: K/O/Mo/L/Ma/H

10. **Cibotiaceae** Korall

Cibotium Kaulf.

[E] ***Cibotium chamissoi*** Kaulf.

Distribution: O/Mo/L/Ma/H

[E] ***Cibotium glaucum*** (Sm.) Hook. & Arn.

Distribution: K/O/Mo/L/Ma/H

[E] ***Cibotium menziesii*** Hook.

Distribution: K/O/Mo/L/Ma/H

[E] ***Cibotium nealiae*** O. Deg.

Distribution: K

11. **Cyatheaceae** Kaulf.

Sphaeropteris Bernh.

[NZ] ***Sphaeropteris cooperi*** (Hook. ex F. Muell.) R. M. Tryon

Distribution: K/O/L/Ma/H

12. **Dicksoniaceae** M.R. Schomb.

Dicksonia L'Hér.

[NZ] ***Dicksonia fibrosa*** Colenso

Distribution: H

13. **Lindsaeaceae** C. Presl ex M.R. Schomb.

Lindsaea Dryand. ex Sm.

[NZ] ***Lindsaea ensifolia*** Sw.

Distribution: K/O/Mo/Ma/H

Lindsaea repens (Bory) Thwaites

[E] var. ***macraeana*** (Hook. & Arn.) C. Chr.

Distribution: K/O/Mo/L/Ma/H

Sphenomeris Maxon

[I] ***Sphenomeris chinensis*** (L.) Maxon

Distribution: K/O/Mo/L/Ma/H

14. **Dennstaedtiaceae** Lotsy

Hypolepis Bernh.

Hypolepis hawaiiensis Brownsey

[E] var. ***hawaiiensis***

Distribution: K/O/Mo/L/Ma/H

[E] var. ***mauiensis*** (Hillebr.) D. D. Palmer

Distribution: Ma

Microlepia C. Presl

[1] ***Microlepia speluncae*** (L.) T. Moore

Distribution: K/O/Mo/Ma/H

Microlepia strigosa (Thunb.) C. Presl

[E] var. ***mauiensis*** (W. H. Wagner) D. D. Palmer

Distribution: Ma/H

[I] var. ***strigosa***

Distribution: K/O/Mo/L/Ma/H

Pteridium Gled. ex Scop.

Pteridium aquilinum (L.) Kuhn

[E] subsp. ***decompositum*** (Gaudich.) Lamoureux ex J. A. Thomson

Distribution: K/O/Mo/L/Ma/H

15. **Pteridaceae** E. D. M. Kirchn.

Adiantum L.

[NZ] ***Adiantum 'Edwinii'***

Distribution: O/L/Ma

[I] ***Adiantum capillus-veneris*** L.

Distribution: N/K/O/Mo/L/Ma/H

[NZ] ***Adiantum hispidulum*** Sw.

Distribution: K/O/Mo/L/Ka/Ma/H

[NZ] ***Adiantum raddianum*** C. Presl

Distribution: K/O/Mo/L/Ma/H

[NZ] ***Adiantum tenerum*** Sw.

Distribution: O/Mo/Ma

Ceratopteris Brongn.

[NZ] ***Ceratopteris gaudichaudii*** Brongn.

var. ***vulgaris*** Masuyama & Watano

Distribution: K/O/H

Cheilanthes Sw.

[NZ] ***Cheilanthes viridis*** (Forssk.) Sw.

Distribution: K/O/L/Ma/H

Coniogramme Fée

[E] ***Coniogramme pilosa*** (Brack.) Hieron.

Distribution: K/O/Mo/L/Ma/H

Doryopteris J. Sm.

[E] ***Doryopteris angelica*** K. R. Wood & W. H. Wagner

Distribution: K

[E] ***Doryopteris decipiens*** (Hook.) J. Sm.

Distribution: N/K/O/Mo/L/Ka/Ma/H

[E] ***Doryopteris decora*** Brack.

Distribution: K/O/Mo/L/Ka/Ma/H

[E] ***Doryopteris takeuchii*** (W. H. Wagner) W. H. Wagner

Distribution: O

Haplopteris C. Presl

[I] ***Haplopteris elongata*** (Sw.) E. H. Crane

Distribution: K/O/Mo/L/Ma/H

Pellaea Link

[I]***Pellaea ternifolia*** (Cav.) Link

Distribution: K/O/Mo/L/Ma/H

Pityrogramma Link

[NZ] ***Pityrogramma austroamericana*** Domin

Distribution: N/K/O/Mo/L/Ka/Ma/H

[NZ] ***Pityrogramma calomelanos*** (L.) Link

Distribution: N/K/O/Mo/L/Ka/Ma/H

Pteris L.

[I]***Pteris cretica*** L.

Distribution: K/O/Mo/L/Ma/H

[I]***Pteris excelsa*** Gaudich.

Distribution: K/O/Mo/L/Ma/H

[E] ***Pteris hillebrandii*** Copel.

Distribution: K/O/Mo/L/Ma/H

[E] ***Pteris irregularis*** Kaulf.

Distribution: K/O/Mo/L/Ma/H

[E] ***Pteris lidgatei*** (Hillebr.) Christ

Distribution: O/Mo/Ma

[NZ] ***Pteris tremula*** R. Br.

Distribution: Ma

[NZ] ***Pteris vittata*** L.

Distribution: K/O/Mo/L/Ma/H

16. **Cystopteridaceae** (Payer) Shmakov

Cystopteris Bernh.

[E] ***Cystopteris douglasii*** Hook.

Distribution: Ma/H

[E] ***Cystopteris sandwicensis*** Brack.

Distribution: K/O/L/Ma

17. **Aspleniaceae** Newman

Asplenium **L.**

[E] ***Asplenium acuminatum*** Hook. & Arn.

Distribution: K/O/Mo/L/Ma/H

[I] ***Asplenium adiantum-nigrum*** L.

Distribution: K/O/Mo/L/Ma/H

[I] ***Asplenium aethiopicum*** (Burm. f.) Bech.

Distribution: K/O/Mo/L/Ma/H

[I] ***Asplenium caudatum*** G.Forst.

Syn. *Asplenium horridum* Kaulf. var. *glabratum* (W. J. Rob.) D. D. Palmer; *Asplenium horridum* Kaulf. var. *horridum*

Distribution: K/O/Mo/L/Ma/H

Asplenium contiguum Kaulf.

[E] var. ***contiguum***

Distribution: K/O/Mo/L/Ma/H

[E] var. ***hirtulum*** C. Chr.

Distribution: K/Ma

[E] ***Asplenium dielerectum*** Viane

Syn. *Diellia erecta* Brack.

Distribution: O/Mo/L (ex)/Ma/H

[E] ***Asplenium dielfalcatum*** Viane

Syn. *Diellia falcata* Brack.

Distribution: O

[E] ***Asplenium diellaciniatum*** Viane

Distribution: K

[E] ***Asplenium dielmannii*** Viane

Syn. *Diellia mannii* (D. C. Eaton) W. J. Rob.

Distribution: K

[E] ***Asplenium dielpallidum*** N. Snow

Syn. *Diellia pallida* W. H. Wagner

Distribution: K

[I] ***Asplenium excisum*** C. Presl

Distribution: K/O/Mo/L/Ma/H

[E] ***Asplenium haleakalense*** W. H. Wagner

Distribution: Ma/H

[E] ***Asplenium hobdyi*** W. H. Wagner

Distribution: K/Mo/Ma/H

[I] ***Asplenium insiticium*** Brack.

Distribution: K/O/Mo/L/Ma/H

[E] ***Asplenium kaulfussii*** Schltdl.

Distribution: K/O/Mo/L/Ma/H

[E] ***Asplenium leucostegioides*** Baker

Syn. *Diellia leucostegioides* (Baker) W. H. Wagner

Distribution: Ma (ex)

[I]***Asplenium lobulatum*** Mett.

Distribution: K/O/Mo/L/Ma/H

[E] ***Asplenium macraei*** Hook. & Grev.

Distribution: K/O/Mo/L/Ma/H

[I]***Asplenium monanthes*** L.

Distribution: Ma/H

Asplenium nidus L.

Distribution: K/O/Mo/L/Ma/H

[I]***Asplenium normale*** D. Don

Distribution: K/O/Mo/L/Ma/H

Asplenium peruvianum Desv.

[E] var. ***insulare*** (C. V. Morton) D. D. Palmer

Distribution: Ma/H

[I]***Asplenium polyodon*** G. Forst.

Distribution: K/O/Mo/L/Ma/H

[E] ***Asplenium schizophyllum*** C. Chr.

Distribution: K/H

[E] ***Asplenium sphenotomum*** Hillebr.

Distribution: K/O/Mo/L/Ma/H

Asplenium trichomanes L.

[E] subsp. ***densum*** (Brack.) W. H. Wagner

Distribution: Ma/H

[I]***Asplenium unilaterale*** Lam.

Distribution: K/O/Mo/L/Ma/H

[E] ***Asplenium unisorum*** (W. H. Wagner) Viane

Syn. *Diellia unisora* W. H. Wagner

Distribution: O

18. **Thelypteridaceae** Ching ex Pic. Serm.

Cyclosorus Link

[E] ***Cyclosorus boydiae*** (D. C. Eaton) W. H. Wagner

Syn. *Christella boydiae* (D.C. Eaton) Holttum

Distribution: O/Ma

[E] ***Cyclosorus cyatheoides*** (Kaulf.) Farw.

Syn. *Christella cyatheoides* (Kaulf.) Holttum

Distribution: K/O/Mo/L/Ma/H

[NZ] ***Cyclosorus dentatus*** (Forssk.) Ching

Syn. *Christella dentata* (Forssk.) Brownsey & Jermy

Distribution: K/O/Mo/L/Ma/H

[E] ***Cyclosorus hudsonianus*** (Brack.) Ching

Syn. *Pneumatopteris hudsoniana* (Brack.) Holttum

Distribution: K/O/Mo/L/Ma/H

[I] ***Cyclosorus interruptus*** (Willd.) H. Itô

Distribution: K/O/Mo/L/Ma/H

[NZ] ***Cyclosorus parasiticus*** (L.) Farw.

Syn. *Christella parasitica* (L.) H. Lév.

Distribution: K/O/Mo/L/Ma/H

[E] ***Cyclosorus pendens*** (D. D. Palmer) N. Snow

Syn. *Pneumatopteris pendens* D. D. Palmer

Distribution: K/O/Mo/Ma/H

[E] ***Cyclosorus sandwicensis*** (Brack.) Copel.

Syn. *Pneumatopteris sandwicensis* (Brack.) Holttum

Distribution: K/O/Mo/L/Ma/H

[E] ***Cyclosorus wailele*** (Flynn) W. H. Wagner

Syn. *Christella wailele* (Flynn) D. D. Palmer

Distribution: K

Macrothelypteris (H. Itô) Ching

[NZ] ***Macrothelypteris torresiana*** (Gaudich.) Ching

Distribution: K/O/Ma/H

Pseudophegopteris Ching

[E] ***Pseudophegopteris keraudreniana*** (Gaudich.) Holttum

Distribution: K/O/Mo/L/Ma/H

Thelypteris Schmidel

[E] ***Thelypteris globulifera*** (Brack.) C. F. Reed

Syn. *Amauropelta globulifera* (Brack.) Holttum

Distribution: K/O/Mo/L/Ma/H

19. **Blechnaceae** Newman

Blechnum L.

[NZ] ***Blechnum appendiculatum*** Willd.

Distribution: K/O/Mo/L/Ma/H

[NZ] ***Blechnum orientale*** L.

Distribution: O

Doodia R. Br.

[E] ***Doodia kunthiana*** Gaudich.

Distribution: K/O/Mo/L/Ma/H

[E] ***Doodia lyonii*** O. Deg.

Distribution: K/O/Ma/H (ex)

Sadleria Kaulf.

[E] ***Sadleria cyatheoides*** Kaulf.

Distribution: K/O/Mo/L/Ma/H

[E] ***Sadleria pallida*** Hook. & Arn.

Distribution: K/O/Mo/L/Ma/H

[E] ***Sadleria souleyetiana*** (Gaudich.) T. Moore

Distribution: K/O/Mo/L/Ma/H

[E] ***Sadleria squarrosa*** (Gaudich.) T. Moore

Distribution: K/O/Mo/L/Ma/H

[E] ***Sadleria unisora*** (Baker) W. J. Rob.

Distribution: K

[E] ***Sadleria wagneriana*** D. D. Palmer & Flynn

Distribution: K

20. **Athyriaceae** Alston

Athyrium Roth

[E] ***Athyrium microphyllum*** (Sm.) Alston

Distribution: K/O/Mo/L/Ma/H

Deparia Hook. & Grev.

[E] ***Deparia cataracticola*** M. Kato

Distribution: K

[E] ***Deparia fenzliana*** (Luerss.) M. Kato

Distribution: K/O/Mo/L/Ma/H

[E] ***Deparia kaalaana*** (Copel.) M. Kato

Distribution: K (ex)/Ma (ex)/H (ex)

[E] ***Deparia marginalis*** (Hillebr.) M. Kato

Distribution: K/Mo/L/Ma/H

[NZ] ***Deparia petersenii*** (Kunze) M. Kato

Distribution: K/O/Mo/L/Ma/H

[E] ***Deparia prolifera*** (Kaulf.) Hook. & Grev.

Distribution: K/O/Mo/L/Ma/H

Diplazium Sw.

[E] ***Diplazium arnottii*** Brack.

Distribution: K/O/Mo/L/Ma/H

[NZ] ***Diplazium esculentum*** (Retz.) Sw.

Distribution: K/O/Mo/L/Ma/H

[E] ***Diplazium molokaiense*** W. J. Rob.

Distribution: K (ex)/O (ex)/Mo (ex)/L (ex)/Ma

[E] ***Diplazium sandwichianum*** (C. Presl) Diels

Distribution: K/O/Mo/L/Ma/H

21. **Dryopteridaceae** Herter

Arachniodes Blume

[E] ***Arachniodes insularis*** W. H. Wagner

Distribution: K/O/Mo/Ma/H

Ctenitis (C. Chr.) C. Chr.

[E] ***Ctenitis latifrons*** (Brack.) Copel.

Distribution: K/O/Mo/L/Ma/H

[E] ***Ctenitis squamigera*** (Hook. & Arn.) Copel.

Distribution: K/O/Mo/L/Ma

Cyrtomium C. Presl

[I] ***Cyrtomium caryotideum*** (Wall. ex Hook. & Grev.) C. Presl

Distribution: K/O/Mo/L/Ma/H

[NZ] ***Cyrtomium falcatum*** (L. f.) C. Presl

Distribution: K/O/Mo/L/Ma/H

Dryopteris Adans.

Dryopteris crinalis (Hook. & Arn.) C. Chr.

[E] var. ***crinalis***

Distribution: K/O/Mo/L/Ma/H

[E] var. ***podosora*** (W. H. Wagner) D. D. Palmer

Distribution: K

Dryopteris fuscoatra (Hillebr.) W. J. Rob.

[E] var. ***fuscoatra***

Distribution: K/O/Mo/L/Ma/H

[E] var. ***lamoureuxii*** Fraser-Jenk.

Distribution: Ma

Dryopteris glabra (Brack.) Kuntze

[E] var. ***alboviridis*** (W. H. Wagner) D. D. Palmer

Distribution: K

[E] var. ***flynnii*** D. D. Palmer

Distribution: K

[E] var. ***glabra***

Distribution: K/O/Mo/L/Ma/H

[E] var. ***hobdyana*** (W. H. Wagner) D. D. Palmer

Distribution: Ma

[E] var. ***nuda*** (Underw.) Fraser-Jenk.

Distribution: K/O/Mo/Ma

[E] var. ***pusilla*** (Hillebr.) Fraser-Jenk.

Distribution: K

[E] var. ***soripes*** (Hillebr.) Herat ex Fraser-Jenk.

Distribution: K/O/Mo/Ma/H

[E] ***Dryopteris hawaiiensis*** (Hillebr.) W. J. Rob.

Distribution: K/O/Mo/Ma/H

[E] ***Dryopteris mauiensis*** C. Chr.

Distribution: K/O/Mo/L/Ma/H

[E] ***Dryopteris rubiginosa*** (Brack.) Kuntze

Syn. *Nothoperanema rubiginosum* (Brack.) A.R. Sm. & D. D. Palmer

Distribution: K/O/Mo/L/Ma/H

[E] ***Dryopteris sandwicensis*** (Hook. & Arn.) C. Chr.

Distribution: K/O/Mo/L/Ma/H

[E] ***Dryopteris subbipinnata*** W. H. Wagner & Hobdy

Distribution: Ma/H

[E] ***Dryopteris tetrapinnata*** W. H. Wagner & Hobdy

Distribution: Ma

Dryopteris unidentata (Hook. & Arn.) C. Chr.

[E] var. ***paleacea*** (Hillebr.) Herat ex Fraser-Jenk.

Distribution: K/O/Mo/Ma/H

[E] var. ***unidentata***

Distribution: K/O/Mo/L/Ma/H

[I]***Dryopteris wallichiana*** (Spreng.) Hyl.

Distribution: K/O/Mo/Ma/H

Elaphoglossum Schott ex J. Sm.

[E] ***Elaphoglossum aemulum*** (Kaulf.) Brack.

Distribution: K/O/Mo/L/Ma/H (ex)

[E] ***Elaphoglossum alatum*** Gaudich.

Distribution: O

[E] ***Elaphoglossum crassicaule*** Copel.

Distribution: K

[E] ***Elaphoglossum crassifolium*** (Gaudich.) W. R. Anderson & Crosby

Distribution: K/O/Mo/L/Ma/H

[E] ***Elaphoglossum fauriei*** Copel.

Distribution: O/Mo

[I]***Elaphoglossum paleaceum*** (Hook. & Grev.) Sledge

Distribution: K/O/Mo/L/Ma/H

[E] ***Elaphoglossum parvisquameum*** Skottsb.

Distribution: Mo/L/Ma/H

[E] ***Elaphoglossum pellucidum*** Gaudich.

Distribution: K/O/Mo/Ma/H

[E] ***Elaphoglossum wawrae*** (Luerss.) C. Chr.

Distribution: K/O/Mo/Ma/H

Polystichum Roth

[E] ***Polystichum bonseyi*** W. H. Wagner & Hobdy

Distribution: Ma/H

[E] ***Polystichum haleakalense*** Brack.

Distribution: Ma/H

[E] ***Polystichum hillebrandii*** Carruth.

Distribution: Ma/H

22. **Nephrolepidaceae** Pic. Serm.

Nephrolepis Schott

[NZ] ***Nephrolepis brownii*** (Desv.) Hovenkamp & Miyam.

Syn. *Nephrolepis multiflora* (Roxb.) F. M. Jarrett ex C. V. Morton

Distribution: N/K/O/Mo/L/Ka/Ma/H

[I]***Nephrolepis cordifolia*** (L.) C. Presl

Distribution: K/O/Mo/L/Ma/H

[E] ***Nephrolepis exaltata*** (L.) Schott subsp. ***hawaiiensis*** W. H. Wagner

Distribution: N/K/O/Mo/L/Ma/H

[NZ] ***Nephrolepis falcata*** (Cav.) C. Chr. ***'Furcans'***

Distribution: K/O/Mo/L/Ma/H

[NZ] ***Nephrolepis hirsutula*** (G. Forst.) C. Presl ***'Superba'***

Distribution: K/Ma

23. **Tectariaceae** Panigrahi

Tectaria Cav.

[E] ***Tectaria gaudichaudii*** (Mett.) Maxon

Distribution: K/O/Mo/L/Ma/H

[NZ] ***Tectaria incisa*** Cav.

Distribution: K/O/Ma/H

24. **Davalliaceae** M. R. Schomb. ex A. B. Frank

Davallia Sm.

[NZ] ***Davallia fejeensis*** Hook.

Distribution: O/Ma

25. **Polypodiaceae** Bercht. & J. Presl

***Adenophorus* Gaudich.**

[E] ***Adenophorus abietinus*** (D. C. Eaton) K. A. Wilson

Distribution: K/O/L/Ma

[E] ***Adenophorus epigaeus*** (L. E. Bishop) W. H. Wagner

Distribution: K

[E] ***Adenophorus haalilioanus*** (Brack.) K. A. Wilson

Distribution: K/O

[E] ***Adenophorus hymenophylloides*** (Kaulf.) Hook. & Grev.

Distribution: K/O/Mo/L/Ma/H

[E] ***Adenophorus oahuensis*** (Copel.) L. E. Bishop

Distribution: O

[E] ***Adenophorus periens*** L. E. Bishop

Distribution: K/O (ex)/Mo/L (ex)/Ma (ex)/H

Adenophorus pinnatifidus Gaudich.

[E] var. ***pinnatifidus***

Distribution: K/O/Mo/L/Ma/H

[E] var. ***rockii*** (Copel.) D. D. Palmer

Distribution: K/O/Mo/Ma

Adenophorus tamariscinus (Kaulf.) Hook. & Grev.

[E] var. ***montanus*** (Hillebr.) L. E. Bishop

Distribution: Mo/Ma/H

[E] var. ***tamariscinus***

Distribution: K/O/Mo/L/Ma/H

[E] ***Adenophorus tenellus*** (Kaulf.) Ranker

Syn. *Grammitis tenella* Kaulf.

Distribution: K/O/Mo/L/Ma/H

[E] ***Adenophorus tripinnatifidus*** Gaudich.

Distribution: K/O/Mo/L/Ma/H

Lepisorus (J. Sm.) Ching

[I] ***Lepisorus thunbergianus*** (Kaulf.) Ching

Distribution: K/O/Mo/L/Ma/H

Microsorum Link

Microsorum spectrum (Kaulf.) Copel.

[E] var. ***pentadactylum*** (Hillebr.) D. D. Palmer

Distribution: K/Ma

[E] var. ***spectrum***

Distribution: K/O/Mo/L/Ma/H

Oreogrammitis Copel.

[E] ***Oreogrammitis baldwinii*** (Baker) Parris

Syn. *Grammitis baldwinii* (Baker) Copel.

Distribution: K

[E] ***Oreogrammitis forbesiana*** (W. H. Wagner) Parris

Syn. *Grammitis forbesiana* W. H. Wagner

Distribution: K/Mo/Ma

[E] ***Oreogrammitis hookeri*** (Brack.) Parris

Syn. *Grammitis hookeri* (Brack.) Copel.

Distribution: K/O/Mo/L/Ma/H

note: Oreogrammitis hookeri was previously thought to be an indigenous species, but is now considered as distinct from species found in Fiji and American Samoa (B. Parris, pers. comm.)

Phlebodium (R. Br.) J. Sm.

[NZ] ***Phlebodium aureum*** (L.) J. Sm.

Distribution: K/O/Mo/L/Ma/H

Phymatosorus Pic. Serm.

[NZ] ***Phymatosorus grossus*** (Langsd. & Fisch.) Brownlie

Distribution: K/O/Mo/L/Ma/H

[NZ] ***Phymatosorus scolopendria*** (Burm. f.) Pic. Serm.

Distribution: Ma

Platycerium Desv.

[NZ] ***Platycerium bifurcatum*** (Cav.) C. Chr.

Distribution: O/Ma/H

[NZ] ***Platycerium superbum*** de Jonch. & Hennipman

Distribution: O

Polypodium L.

Polypodium pellucidum Kaulf.

[E] var. ***acuminatum*** D. D. Palmer

Distribution: K/Ma

[E] var. ***pellucidum***

Distribution: K/O/Mo/L/Ma/H

[E] var. ***vulcanicum*** Skottsb.

Distribution: Mo/Ma/H

Pyrrosia Mirb.

[NZ] ***Pyrrosia piloselloides*** (L.) M. G. Price

Distribution: O

Stenogrammitis Labiak

[E] ***Stenogrammitis saffordii*** (Maxon) Labiak

Syn. *Lellingeria saffordii* (Maxon) A. R. Sm. & R. C. Moran

Distribution: K/O/Mo/L/Ma/H

APPENDIX 2. Estimated origins of 78 Hawaiian fern and lycophyte taxa based on molecular phylogenetic studies and/or geographical distributions.

[E] endemic; [I] indigenous
* = origin inferred based on distribution of genus or species

Status	Taxa	Distribution of lineage sister to Hawaiian Lineage	References
	Ferns		
E	*Adenophorus* - 10 species	Neotropics	Ranker *et al.*, 2004
E	*Arachniodes insularis*	Asia	*
E	*Asplenium acuminatum*	unknown	
E	*Asplenium contiguum*	unknown	
E	*Asplenium haleakalense*	unknown	
E	*Asplenium hobdyi*	Paleotropics	Schneider *et al.*, 2005
E	*Asplenium kaulfussii*	unknown	
E	*Asplenium leucostegioides*	unknown	
E	*Asplenium macraei*	unknown	
E	*Asplenium peruvianum* var. *insulare*	Neotropics	*
E	*Asplenium schizophyllum*	unknown	
E	*Asplenium sphenotomum*	unknown	
E	*Asplenium trichomanes* var. *densum*	Cosmopolitan	*
E	*Asplenium unisorum*	unknown	
E	*Athyrium microphyllum*	unknown	
E	*Callistopteris baldwinii*	Asia/Indopacific	*
E	*Cibotium* - 4 species	Neotropics	Geiger *et al.*, 2007
E	*Coniogramme pilosa*	Asia/Indopacific	*
E	*Crepidomanes draytonianum*	unknown	
E	*Ctenitis latifrons*	unknown	
E	*Ctenitis squamigera*	unknown	
E	*Cyclosorus boydiae*	unknown	
E	*Cyclosorus cyatheoides*	unknown	
E	*Cyclosorus hudsonianus*	unknown	
E	*Cyclosorus pendens*	unknown	
E	*Cyclosorus sandwicensis*	unknown	
E	*Cyclosorus wailele*	unknown	
E	*Cystopteris douglasii*	unknown	
E	*Cystopteris sandwicensis*	unknown	
E	*Deparia cataracticola*	Asia	*
E	*Deparia fenzliana*	Asia	*
E	*Deparia kaalaana*	unknown	
E	*Deparia marginalis*	unknown	
E	*Deparia prolifera*	unknown	
E	*Diellia* clade of *Asplenium* (6 spp.)	Paleotropics	Schneider *et al.*, 2005
E	*Diplazium arnottii*	unknown	
E	*Diplazium molokaiense**	unknown	
E	*Diplazium sandwichianum*	unknown	
E	*Diplopterygium pinnatum*	Asia/Indopacific	Ranker *et al.*, unpub. data
E	*Doodia kunthiana*	Indopacific	*
E	*Doodia lyonii*	Indopacific	*
E	*Doryopteris angelica*	unknown	
E	*Doryopteris decipiens*	unknown	

APPENDIX 2. Continued.

Status	Taxa	Distribution of lineage sister to Hawaiian Lineage	References
E	*Doryopteris decora*	unknown	
E	*Doryopteris subdecipiens*	unknown	
E	*Doryopteris takeuchii*	unknown	
E	*Dryopteris crinalis, D. mauiensis, D. sandwicensis, D. tetrapinnata, D. unidentata*	Asia	Geiger and Ranker, 2005
E	*Dryopteris fusco-atra*	Asia	Geiger and Ranker, 2005
E	*Dryopteris glabra + D. hawaiiensis*	Asia	Geiger and Ranker, 2005
E	*Dryopteris rubiginosa*	Asia	Geiger and Ranker, 2005
E	*Dryopteris subbipinnata* (sister to *D. wallichiana*)	Pantropical	Geiger and Ranker, 2005
E	*Elaphoglossum aemulum*	unknown	
E	*Elaphoglossum alatum*	unknown	
E	*Elaphoglossum crassicaule*	unknown	
E	*Elaphoglossum crassifolium*	unknown	
E	*Elaphoglossum fauriei*	unknown	
E	*Elaphoglossum parvisquameum*	unknown	
E	*Elaphoglossum pellucidum*	unknown	
E	*Elaphoglossum wawrae*	unknown	
E	*Hymenophyllum lanceolatum*	Indopacific	Ebihara *et al.*, 2004; Hennequin *et al.*, 2003, 2006; Pryer *et al.*, 2001
E	*Hymenophyllum obtusum*	unknown	
E	*Hymenophyllum recurvum*	unknown	
E	*Hypolepis hawaiiensis*	Austral	Ranker *et al.*, unpub. data
E	*Lindsaea repens* var. *macraeana*	Asia/Indopacific	*
E	*Marattia douglasii*	Neotropics	Murdock, 2008
E	*Marsilea villosa*	North America/ Neotropics	Whitten *et al.*, 2012
E	*Microsorum spectrum*	Asia	Kreier *et al.*, 2008
E	*Nephrolepis exaltata* subsp. *hawaiiensis*	Pantropical	*
E	*Oreogrammitis baldwinii, O. forbesiana, O. hookeri*	Asia/Indopacific	Ranker *et al.*, 2004
E	*Polypodium pellucidum*	Boreal	Haufler and Ranker, 1995
E	*Polystichum bonseyi + P. haleakalense*	Asia	Driscoll and Barrington, 2007
E	*Polystichum hillebrandii*	Asia	Driscoll and Barrington, 2007
E	*Pseudophegopteris keraudreniana*	Asia	*
E	*Pteris hillebrandii*	unknown	
E	*Pteris irregularis*	unknown	
E	*Pteris lydgatei*	unknown	
E	*Sadleria* - 6 species	unknown	
E	*Sceptridium subbifoliatum**	unknown	
E	*Schizaea robusta*	unknown	
E	*Stenogrammitis saffordii*	Neotropics	Sundue *et al.*, 2010
E	*Sticherus owhyhensis*	unknown	
E	*Tectaria gaudichaudii*	unknown	
E	*Thelypteris globulifera*	unknown	
E	*Vandenboschia cyrtotheca*	unknown	

APPENDIX 2. Continued.

Status	Taxa	Distribution of lineage sister to Hawaiian Lineage	References
E	*Vandenboschia davallioides*	unknown	
E	*Vandenboschia tubiflora*	unknown	
I	*Adiantum capillus-veneris*	Cosmopolitan	*
I	*Asplenium adiantum-nigrum*	Paleotropics	*
I	*Asplenium aethiopicum*	Pantropical	*
I	*Asplenium caudatum*	Asia/Indopacific	*
I	*Asplenium excisum*	Asia/Indopacific	*
I	*Asplenium insiticium*	Indopacific	*
I	*Asplenium lobulatum*	Asia/Indopacific	*
I	*Asplenium monanthes*	Pantropical	*
I	*Asplenium nidus*	Paleotropics	*
I	*Asplenium normale*	Paleotropics	*
I	*Asplenium polyodon*	Paleotropics	*
I	*Asplenium unilaterale*	Paleotropics	*
I	*Crepidomanes minutum*	Asia	*
I	*Crepidomanes proliferum*	Asia/Indopacific	*
I	*Cyclosorus interruptus*	Pantropical	*
I	*Cyrtomium caryotideum*	Asia	*
I	*Dicranopteris linearis*	Paleotropics	*
I	*Dryopteris wallichiana*	Pantropical	*
I	*Elaphoglossum paleaceum*	Neotropics	*
I	*Haplopteris elongata*	Paleotropics	*
I	*Lepisorus thunbergianus*	Asia/Indopacific	*
I	*Lindsaea repens*	Paleotropics	*
I	*Microlepia speluncae*	Paleotropics	*
I	*Microlepia strigosa*	Paleotropics	*
I	*Nephrolepis cordifolia*	Pantropical	*
I	*Ophioderma pendulum*	Paleotropics	*
I	*Ophioglossum nudicaule*	Pantropical	*
I	*Ophioglossum petiolatum*	Pantropical	*
I	*Ophioglossum polyphyllum*	Paleotropics	*
I	*Pellaea ternifolia*	Neotropics	*
I	*Psilotum complanatum*	Pantropical	*
I	*Psilotum nudum*	Pantropical	*
I	*Pteridium aquilinum*	North America	Der *et al.*, 2009
I	*Pteris cretica*	Cosmopolitan	*
I	*Pteris excelsa*	Paleotropics	*
I	*Sphenomeris chinensis*	Paleotropics	*
I	*Tectaria incisa*	Neotropics	*
	Lycophytes		
E	*Huperzia erosa*	unknown	
E	*Huperzia mannii*	unknown	
E	*Huperzia nutans*	unknown	
E	*Huperzia stemmermanniae*	unknown	
E	*Huperzia subintegra*	unknown	
E	*Isoëtes hawaiiensis*	Boreal	Hoot *et al.*, 2006
E	*Selaginella deflexa*	unknown	
I	*Selaginella arbuscula*	South Pacific	*
I	*Huperzia erubescens*	Asia	*

APPENDIX 2. Continued.

Status	Taxa	Distribution of lineage sister to Hawaiian Lineage	References
I	*Huperzia filiformis*	Tropical America	*
I	*Huperzia haleakalae*	Boreal	*
I	*Huperzia phyllantha*	Asia/Indopacific	*
I	*Huperzia serrata*	Asia	*
I	*Lycopodiella cernua*	Pantropical	*
I	*Lycopodium venustulum*	South Pacific	*

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Myriopteris windhamii sp. nov., a New Name For *Cheilanthes villosa* (Pteridaceae)

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Abstract.—Recent studies examining the evolutionary relationships of species assigned to *Cheilanthes* (Pteridaceae) reveal that the genus is highly polyphyletic. To achieve a monophyletic generic classification of the 400+ taxa of cheilanthoid ferns, it is necessary to transfer species that are only distantly related to the type species (*Cheilanthes micropteris*) to other genera. One of these species is *Cheilanthes villosa* Davenp. ex Maxon, which needs to be reassigned to the genus *Myriopteris*. Because the epithet *villosa* is preoccupied in *Myriopteris* and there are no synonyms for this distinctive taxon, a new name is required. The species is herein renamed *Myriopteris windhamii*.

Key Words.—apomixis, cheilanthoid, myriopterid, triploid

Recent molecular systematic studies of cheilanthoid ferns (Pteridaceae) have confirmed that the globally distributed genus *Cheilanthes* Sw. is highly polyphyletic (Eiserhardt *et al.* 2011; Gastony and Rollo 1998; Li *et al.* 2012; Link-Perez *et al.* 2011; Prado *et al.* 2007; Rothfels *et al.* 2008; Schuettpelz *et al.* 2007; Windham *et al.* 2009; Zhang *et al.* 2007). Species currently assigned to *Cheilanthes* occur in five of the six major cheilanthoid clades identified by Windham *et al.* (2009) and Eiserhardt *et al.* (2011). To achieve a monophyletic generic classification of the 400+ taxa of cheilanthoid ferns that includes more than a single genus, it is necessary to transfer to other genera those species that are only distantly related to the South American generitype, *Cheilanthes micropteris* Sw. A series of recent papers (e.g., Li *et al.* 2012; Perez-Link *et al.* 2011; Yatskievych and Arbeláez 2008) have initiated this process of narrowing and refining the circumscription of *Cheilanthes*. Continuing this effort, we are preparing to resurrect the genus *Myriopteris* Fée (Grusz *et al.* in prep), which, in its emended form, will include most of the North American taxa currently residing in *Cheilanthes*.

The clade that will become *Myriopteris* Fée *emend* Grusz & Windham is a well supported monophyletic group that comprises roughly 10% of cheilanthoid species diversity. It is among the earliest diverging lineages of cheilanthoid ferns and only distantly related to the type species of *Cheilanthes*. Most of the 40+ species in this group are easily transferred from *Cheilanthes* to *Myriopteris*, the most obvious exception being *Cheilanthes villosa* Davenp. ex Maxon. The name *Myriopteris villosa* was previously published by Fée (1852) based on a collection now associated with *Cheilanthes lendigera* (Cav.) Sw. (= *Myriopteris lendigera* (Cav.) Fée). Because the epithet *villosa* is thus preoccupied in *Myriopteris* and there are no synonyms for the

distinctive taxon known as *Cheilanthes villosa*, a new name is required. This apomictic triploid species is here renamed *Myriopteris windhamii*.

Myriopteris windhamii Grusz, ***sp. nov.*** TYPE.—USA. Arizona: Cochise Co., SSW of Sierra Vista in the Huachuca Mts. along Copper Canyon, ca. 0.25 trail miles NE of its intersection with W Montezuma Canyon Road. Lat./Long/: 31.36385N 110.29794W (WGS84 Datum). Elevation 6175 feet. 16 August 2013, *Windham 4165* (holotype: DUKE; isotypes: ARIZ, ASC, ASU, GH, MO, NMC, NY, TEX/LL, UNM, US, UT). **Figs. 1–2.**

Cheilanthes villosa Davenp. ex Maxon, Proc. Biol. Soc. Wash. 31: 142. 1918; non *Myriopteris villosa* Fée

Rhizomes compact, scales linear-lanceolate, entire to slightly erose, 0.2–0.6 mm wide, often with a well defined, lustrous, dark central stripe and light brown margins, with tufted concolorous scales at rhizome apices and stipe bases, straight to slightly contorted, loosely appressed, persistent; *leaves* clustered, 7–30 cm long, with non-circinate vernation; *blades* narrowly oblong-lanceolate, 3–4-pinnate at the base; *petioles* dark reddish brown, lustrous, terete, with scattered, ascending, lanceolate scales, largely paleaceous, darker at point of attachment, mixed with abundant, hair-like scales; *rachises* with mostly hair-like scales adaxially and broader lanceolate to ovate scales abaxially, otherwise similar to petioles; *pinnae* 8–20 pairs, not articulate, with dark color of rachises continuing into pinna bases, basal pair usually slightly smaller than adjacent pair, equilateral, appearing villous above and scaly below; *costae* adaxial surface greenish except at base, with scattered hairs and hair-like scales, abaxial surface of costae dark and lustrous except at apex, with conspicuous ovate-lanceolate, truncate to shallowly cordate scales, these 0.7–1.2 mm wide, strongly imbricate, spreading laterally, with erose-dentate margins, not ciliate, brown proximally, paleaceous distally, attenuate to hair-like apices; *ultimate segments* round to elliptical, the largest 1–2 mm wide, with coarse, contorted, whitish or translucent hairs adaxially and occasional hairs and narrow scales abaxially; *leaf margins* recurved, forming a weakly differentiated marginal false indusium to 0.2 mm wide; *sporangia* attached near vein endings, becoming confluent at maturity, partially covered by false indusium; *spores* 32 per sporangium, 60.8 ± 2.47 μm in diameter; $n = 2n = 90II$ (apomictic; Windham and Yatskievych 2003).

Paratypes.—**U.S.A. Arizona:** Cochise Co., west wall of Copper Canyon in the Huachuca Mts. ca. 2.42 km NNW of the summit of Coronado Peak, Coronado National Forest, Montezuma Pass Quad (7 ½ min.), UTM - 3469960 m. N by 566775 m. E (Zone 12), elevation 6250 ft., 5 August 1983, *Windham 458*. Pima Co., Waterman Mts., N-facing slope on NE end of range, T12S R8E S24, pitted limestone with *Larrea, Cereus giganteus, Cercidium*, 3200 ft., *Bruce Parfitt 2786*, 24 Mar 1979 (NY); Cochise Co., Coronado National Forest, Huachuca Mts., east side of Copper Canyon at a point 3.04 km. NNW of the summit of Coronado Peak, Montezuma Pass Quadrangle (7 ½ min.), Universal Transverse

FIG. 1. Scanned image of the holotype of *Myriopteris windhamii* (*Windham 458*, DUKE). Scale bar in centimeters.

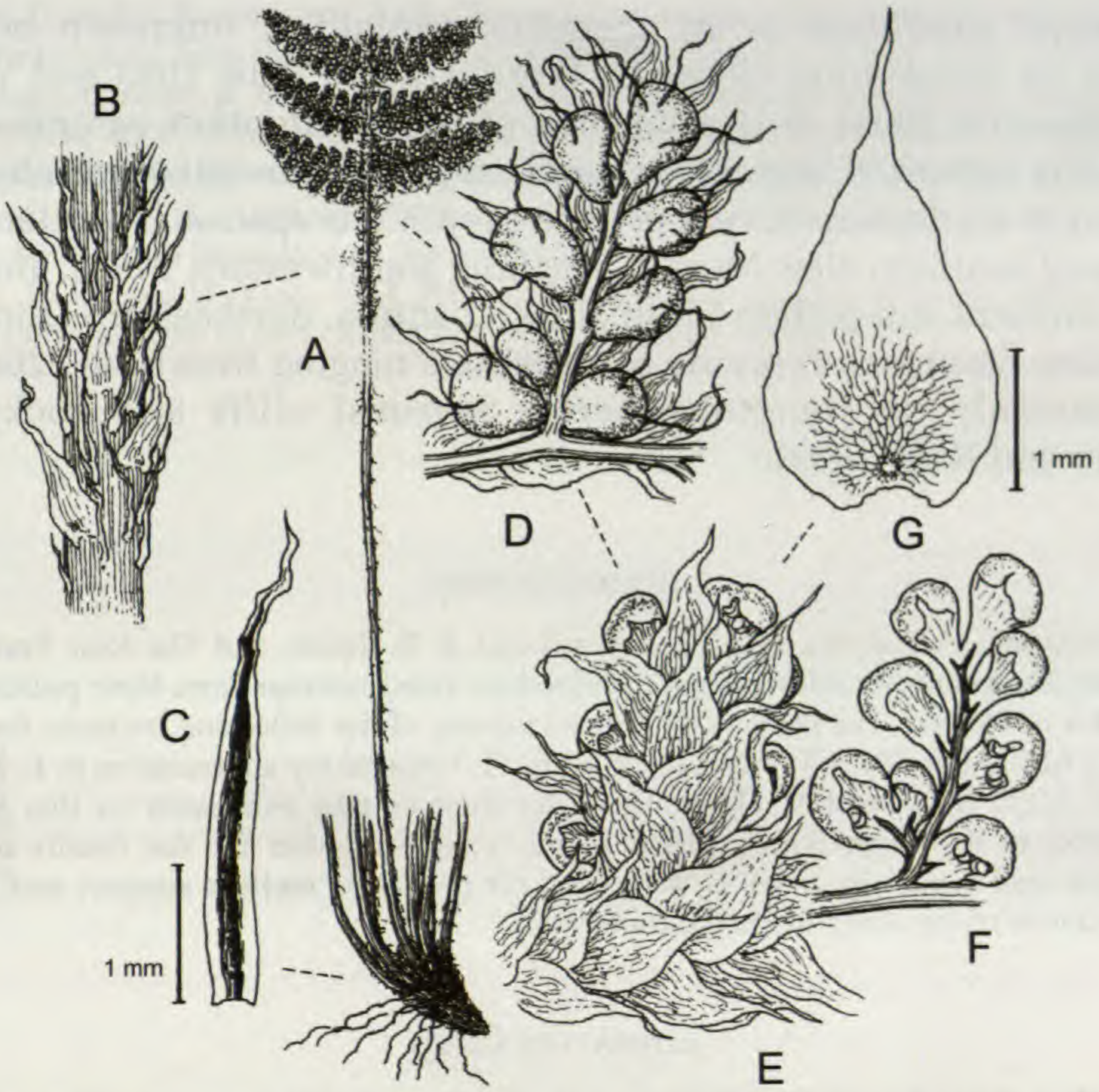

FIG. 2. *Myriopteris windhamii* (*Pringle 459*, NY). A. Rhizome and partial frond. B. Stipe detail. C. Rhizome scale. D. Pinnule, adaxial surface. E. Pinnule, abaxial surface. F. Pinnule, abaxial surface, with scales removed. G. Blade scale. Scale bars = 1 mm. Reproduced from *The Pteridophytes of Mexico* (Mickel and Smith, 2004) with permission of The New York Botanical Garden Press.

Mercator- 3470625m.N by 566650m.E (Zone 12), growing from crack under overhang on cliff face ca. 6 meters E. of streambed, about 25° facing west, locally common on limestone outcrops at lower elevations., associated genera *Quercus, Brickellia, Pinus, Garrya, Cercocarpus,* and *Juniperus, Windham 0236*, 17 March 1981 (UT, ASC). **New Mexico:** Eddy Co., Lincoln National Forest, along rd. 409 to Sitting Bull Falls, open NW facing (309°) limestone cliff with *Quercus, Dasylirion, Opuntia, Rhus, Gutierrezia*, N 32.2630, W 104.6807, 1356 m. *Beck 1050*, 02 May 2008 (NY, DUKE). **Texas:** El Paso Co., Tom Mays County Park, Franklin Mountain, northwest edge of El Paso, common under rocks in ravine on limestone hillside in Chihuahuan Desert, with *Notholaena, Cheilanthes, Pellaea, Agave, Yucca, Dasylirion, Quercus,* and grasses, elevation 4400 feet, *Wollenweber & Yatskievych 81-510*, 15 December 1981 (ARIZ).

Etymology.—This name honors Dr. Michael D. Windham, acknowledging his lifelong dedication to the study of cheilanthoid ferns and, in particular, his devotion to understanding the evolution and cytogenetics of apomictic polyploids (including his new namesake in *Myriopteris*).

Myriopteris windhamii is an apomictic triploid of unknown origin (see comments on *Cheilanthes villosa* by Windham and Rabe 1993 and Windham and Yatskievych 2003). It is a member of the *Cheilanthes eatonii* complex, along with *C. eatonii*, *C. tomentosa*, and *C. myriophylla*—all of which are being transferred to *Myriopteris* (Grusz *et al.* in prep.). The species is scattered across Arizona and southern New Mexico, reaching southwestern Texas, and, within Mexico, northern and eastern Chihuahua, Coahuila, northeastern Durango and northeastern Zacatecas. It occurs at elevations ranging from 400–2200 meters, most commonly on limestone (rarely igneous) cliffs and rocky slopes (Windham and Rabe 1993).

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Polystichum montevidense Demystified: Molecular and Morphological Data Reveal a Cohesive, Widespread South American Species

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Abstract.—The genus *Polystichum* presents striking variation in morphology and habitat preference in the Central Andes, the Serra do Mar, and adjacent regions. Among these taxa, *Polystichum montevidense* is a name long applied to an array of twice-pinnate species with dark petiole scales, broad leaves, and no vegetative propagules. Using a classical morphological approach combined with inferences gleaned from molecular data, we analyzed *P. montevidense* and its widespread and ecologically prominent array of allies. Results from our combined molecular and morphological analysis suggest the name *P. montevidense* should be applied to collections from the Central Andes south to Argentina, and east to Uruguay and the southernmost portion of Brazil. Most Brazilian plants determined as *P. montevidense* in herbaria are *P. platylepis*.

Key Words.—Dryopteridaceae, South America, Andes, Brazil, biogeography, phylogeny

Polystichum (Dryopteridaceae) is a monophyletic genus with the inclusion of a few satellite genera and part of *Cyrtomium* (Little and Barrington, 2003; Li *et al.*, 2007). One of the largest fern genera (between 200 and 300 species; Little and Barrington, 2003), it is found worldwide mainly in tropical and warm temperate areas. Its two centers of greatest diversity are in montane forests of warm temperate eastern Asia (Zhang and Barrington, in press) and tropical America (Barrington, 2011). Studies on *Polystichum* in the New World have been focused outside of South America; the main contributions address the genus in Western North America (Wagner, 1979), the West Indies (Mickel 1997, Morejón and Sánchez, 2012), and Mesoamerica (Barrington 1985a, 1985b, 1989, 1990, 1992, 1995, 2003, 2005, 2011).

Though *Polystichum* itself is morphologically distinct, species delimitation has been a problem for a very long time (e.g., Christensen, 1905–1906; Domin, 1929). This taxonomic problem can be found across all of the centers of diversity for the genus. For instance, Fraser-Jenkins (1997) accepted only one of the 11 *Polystichum* species described in the previous 15 years for the Indo-Himalayan region; he considered the remainder to be synonyms.

In South America the level of confusion is similar, and the name *Polystichum montevidense* emerges as perhaps the most misapplied on the continent (Kessler *et al.* 2005). The taxonomic confusion surrounding this species begins with its brief and vague description by Sprengel (1827). Baker (1870) in *Flora Brasiliensis*, placed it in synonymy with *Polystichum aculeatum* (L.) Roth var. β *phegopteroideum* Baker, a name that included a broad array of Andean exindusiate polystichums. Fée (1869, 1872–1873) in his major work on the Brazilian ferns, did not even mention *P. montevidense*, though he recognized 14 species in *Polystichum* s.l. including ten newly described by him.

Almost a century after its original description, Rosenstock (1906) combined Sprengel's *Polypodium montevidense* into *Polystichum*. Rosenstock's conception of the species was very broad, including as synonyms six of the species described by Fée from the Serra do Mar region of tropical Brazil.

Sehnem (1979), in the most recent flora addressing *Polystichum* diversity in Brazil, rejected Rosenstock's (1906) species circumscriptions and returned to Fée's concept of the genus. In his treatment, following Fée, Sehnem excluded *P. montevidense* entirely. Nevertheless, the name *Polystichum montevidense* has remained in widespread use in Brazil; it is the most common name on Brazilian *Polystichum* specimens, being used extensively for mid-sized 2-pinnate plants with dark, matte-textured petiole-base scales.

Further complicating the situation was the broad application, by Tryon and Stolze (1991), of *P. montevidense* (with two varieties, var. *montevidense* and var. *nudicaule* (Rosenst.) Tryon), to plants distributed from Venezuela to Bolivia and Argentina as well as Brazil and Uruguay. Kessler *et al.* (2005) also recognized a broadly circumscribed *P. montevidense*. They alluded to morphological differences between Bolivian and Southeastern Brazilian plants historically placed in *P. montevidense*, but they did not distinguish them taxonomically. Neither Tryon and Stolze (1991) nor Kessler *et al.* (2005) located Sprengel's type of *P. montevidense*; thus critical study of authentic material for *Polystichum montevidense*, using modern morphological analysis, has not been possible.

In this paper we seek resolution of the long-standing problems surrounding the name *Polystichum montevidense*. Our approach has been to relocate the collections on which the name was based, to collect material for molecular and morphological study from the presumptive type locality north of Montevideo, Uruguay, to perform cytological analysis of the species, to do a molecular phylogenetic analysis of the relevant related *Polystichum* species, and to review the morphological characters of *P. montevidense* and related taxa. This combined morphological, cytological, and molecular analysis yields clear

insights into the species biology and taxonomy of this long-misunderstood taxon.

Methods

Taxon sampling.—Our sample consists of 17 *Polystichum* accessions from across the northern and central Andean region, Argentina, Uruguay, and Brazil. We sampled all taxa shown to have close evolutionary relationships to *P. montevidense* in the phylogeny of McHenry (2012). Also included are three accessions from the senior author's dense sampling of the Serra do Mar region, and one accession of *P. montevidense* from the tropical Andes, one from Argentina, and one from Uruguay, to adequately represent the morphological diversity of the group across its range. We excluded accessions that showed signs of hybridity (intermediate morphology and misshapen spores). Voucher data for all of our collections are listed in the Appendix.

DNA extraction, amplification and sequencing.—Total genomic DNA was extracted from fresh (0.1g) or silica-dried (0.02g) material. Leaf material collected in the field was preserved fresh at 4°C or in silica desiccant gel and stored at −80°C until extraction. Total genomic DNA was extracted from pinnules following a modified CTAB protocol (Doyle and Doyle, 1987). Four plastid DNA regions were amplified using by PCR: two genes (*rps4* and *rbcL*) and two intergenic spacers (the region between *trnL* and *trnF* (*trnLF*) and the region between *trnS* and *rps4* (*trnS-rps4*)). Primers for amplification and sequencing were taken from the literature: *rbcL* (Little and Barrington, 2003), *rps4* (Shaw *et al.*, 2005), *trnLF* (Taberlet *et al.*, 1991), and *trnS-rps4* (Souza-Chies *et al.*, 1997). PCR amplification was performed in a TC-312 or TC-3000 thermal cycler (Techne, Burlington, New Jersey, USA) following protocols in McKeown *et al.* (2012). PCR products were cleaned using ExoSAP-IT (USB Corporation, Cleveland, OH, USA). Sequencing of the cleaned PCR products employed a cycle-sequence reaction using the BigDye Terminator Cycle Sequence Ready Reaction Kit v. 3.1 (Perkin–Elmer/Applied Biosystems, Foster City, CA, USA). Sequences were resolved on an ABI Prism 3100-Avant Genetic Analyzer (Vermont Cancer Center DNA Analysis Facility, Burlington, VT, USA). Consensus sequences from the raw chromatographs (using both the forward and reverse reads) were assembled for each gene using Sequencher 4.5 (Genes Code Corporation, Ann Arbor, MI, USA) or Geneious Pro v.5.0.3 (Drummond *et al.*, 2007).

Sequence alignment and phylogenetic analysis.—Consensus sequences were aligned with MUSCLE (Edgar, 2004) as implemented in Geneious Pro v.5.0.3 (Drummond *et al.*, 2007). All phylogenetically informative indels were coded following the simple gap coding of Simmons and Ochoterena (2000) and added as additional binary characters at the end of the NEXUS file. The concatenated sequences were analyzed by Bayesian inference (BI) using MrBayes v. 3.2 (Ronquist *et al.*, 2012). The data were partitioned by plastid region, and optimal models (Table 1) were applied to each of the molecular partitions. The model selection was done using jModelTest v. 2.1 under the Akaike

TABLE 1. Characteristics of the markers used in the phylogenetic analysis.

Marker	Model	Length (bp)	Variable sites	Sampled taxa
rbcL	K80+G	1165	33 (2.8%)	17
trnLF	TPM2uf+G	331	29 (8.8%)	17
rps4	TPM3uf+G	450	25 (5.6%)	17
trnS-rps4	TrN+G	460	50 (10.9%)	17

Information Criterion (AIC; Darriba *et al.* 2012). We employed a mixed-model Bayesian analysis with different models allowed for each partition. The parameters for each data partition were able to vary freely. Indels were treated as single, independent, and binary characters. BI was preformed in Mr. Bayes by running two independent analyses for 5 million generations with trees sampled every 1,000 generations. Stationarity was calculated by plotting the log-likelihood scores for each run against generation in the program Tracer v1.5 (Rambault and Drummond, 2007). All trees prior to this point (the first 500,000) were discarded as the burn-in phase and a 50% majority rule consensus tree was calculated for the remaining trees.

Chromosome analysis.—Sporophytes collected from wild populations from Córdoba province, Argentina, were cultivated at the Universidad de Córdoba; voucher specimens were deposited at CORD. For the analysis of mitotic chromosomes, croziers were excised into small fragments approximately 2 mm wide. The fragments were treated in a solution of 2mM 8-hydroxiquinoline for 1 h at room temperature, followed by 8 h at 14°C, then fixed in ethanol-acetic acid (3:1) and stored at −20°C. For chromosome analysis, the crozier fragments were washed in distilled water 4 to 5 times, then hydrolyzed in 3 ml of cellulase 2%-pectinase 20%, for 2 h at 37°C. The hydrolyzed cells were stained in alcoholic hydrochloric acid-carmine and squashed following established protocols (Manton, 1950).

RESULTS

Molecular analysis.—The analysis of South American accessions relevant to the disposition of the name *P. montevidense* yielded a highly resolved phylogeny (Fig. 1). In this phylogeny, our accession from the area of the type collection near Montevideo, Uruguay, resolves in a clade with Argentine and Bolivian accessions of *P. montevidense* (Clade 2; posterior probability [PP]=1.0). This trio of accessions is sister to a clade of Andean plants including *P. solomonii* and *P. albomarginatum*. Together they form a well-supported clade (PP=0.96) with *P. opacum*, the Brazilian representative of the lineage that includes the widespread *P. platyphyllum*. In contrast, our accessions of specimens matching plants commonly determined as *P. montevidense* from the northern Serra do Mar, Brazil, resolve in a well-supported clade (Clade 1, PP=1.0) with the tropical Southeast Brazilian endemic species *P. platylepis* and *P. pallidum* (Condack, 2012).

Morphological analysis.—We found that the Sello collections of *Polypodium montevidense* from near Montevideo, Uruguay (Brazil in Sello's time), which

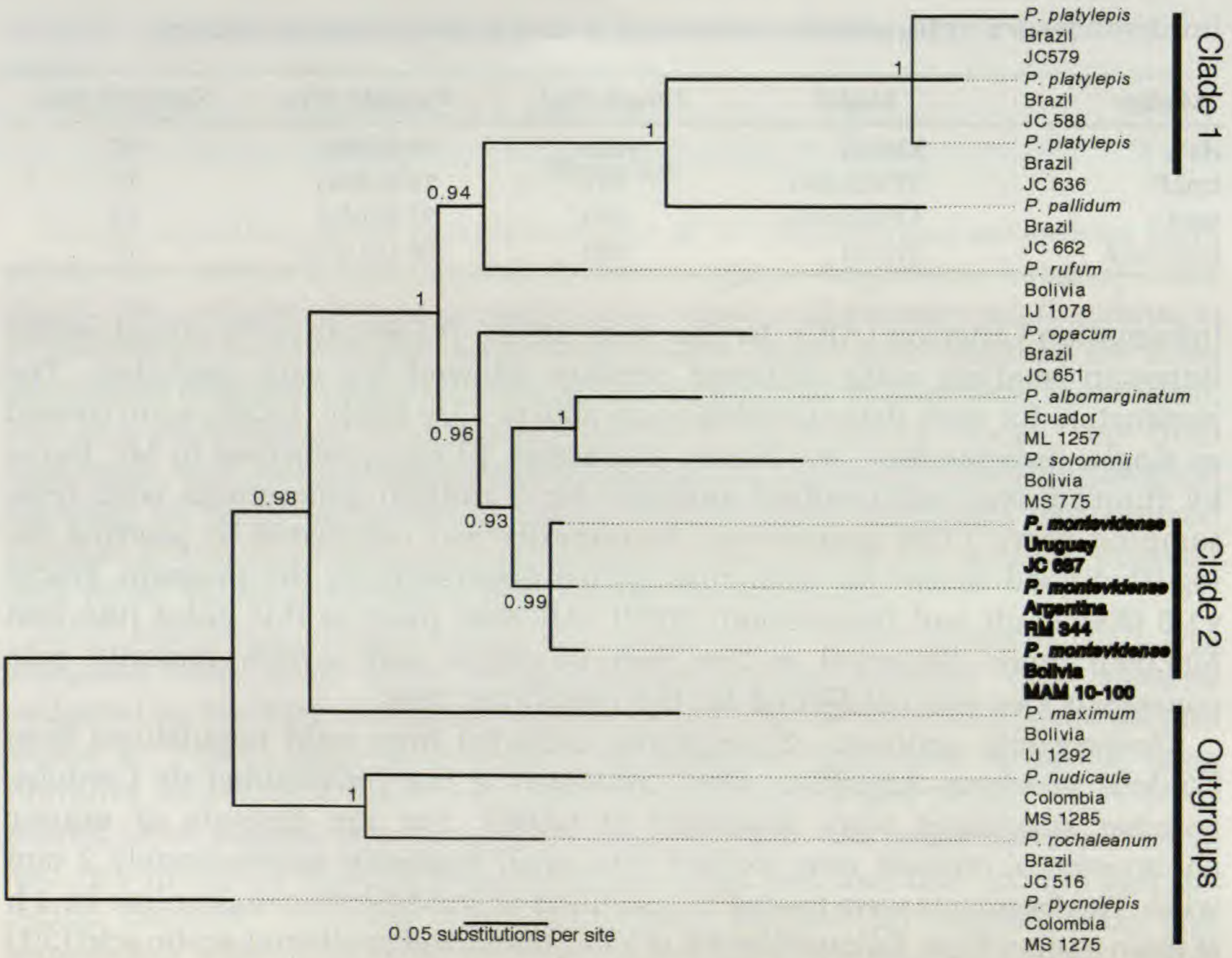

FIG. 1. Phylogeny of *Polystichum montevidense* and its nearest allies. The tree is the 50% majority rule phylogram from the Bayesian inference analysis. Posterior probabilities are given at each node. *Polystichum montevidense* accessions are in bold. Country of each accession, collector initials, and collection number are below each accession (see Appendix).

survive at Berlin, include six sheets numbered *d. 654*. One of these (B 20 0156392) is determined as *Polypodium montevidense* in a hand annotated as Sprengel's. These specimens, which we take to be syntypes of *Polystichum montevidense*, are morphologically cohesive, allowing a clear morphological circumscription of the taxon. Additionally, a specimen deposited in the Gaudichaud herbarium at Paris (P 01456828) has a field label attached at the petiole with the same "d 654" found on the original material at B. From these materials, we diagnose the species as having ferruginous stem scales that contrast with the large basal petiole-scales, which have atropurpureous centers and rufous edges; long, delicate petiole-scale cilia; 2-pinnate laminae that lack a bulbil; lanceolate rachis scales that are either entirely rufous or bicolorous with dark centers; long-lanceolate, rufous pinna-rachis scales; serrate pinnules with strongly developed spinules; glandular trichomes on both sides of the pinnules, and no true indusium (Figs. 2, 3). The glandular trichomes are especially significant as they are extraordinarily rare in *Polystichum*, so far known only from juvenile plants of Andean *Polystichum* species (McHenry, 2012).

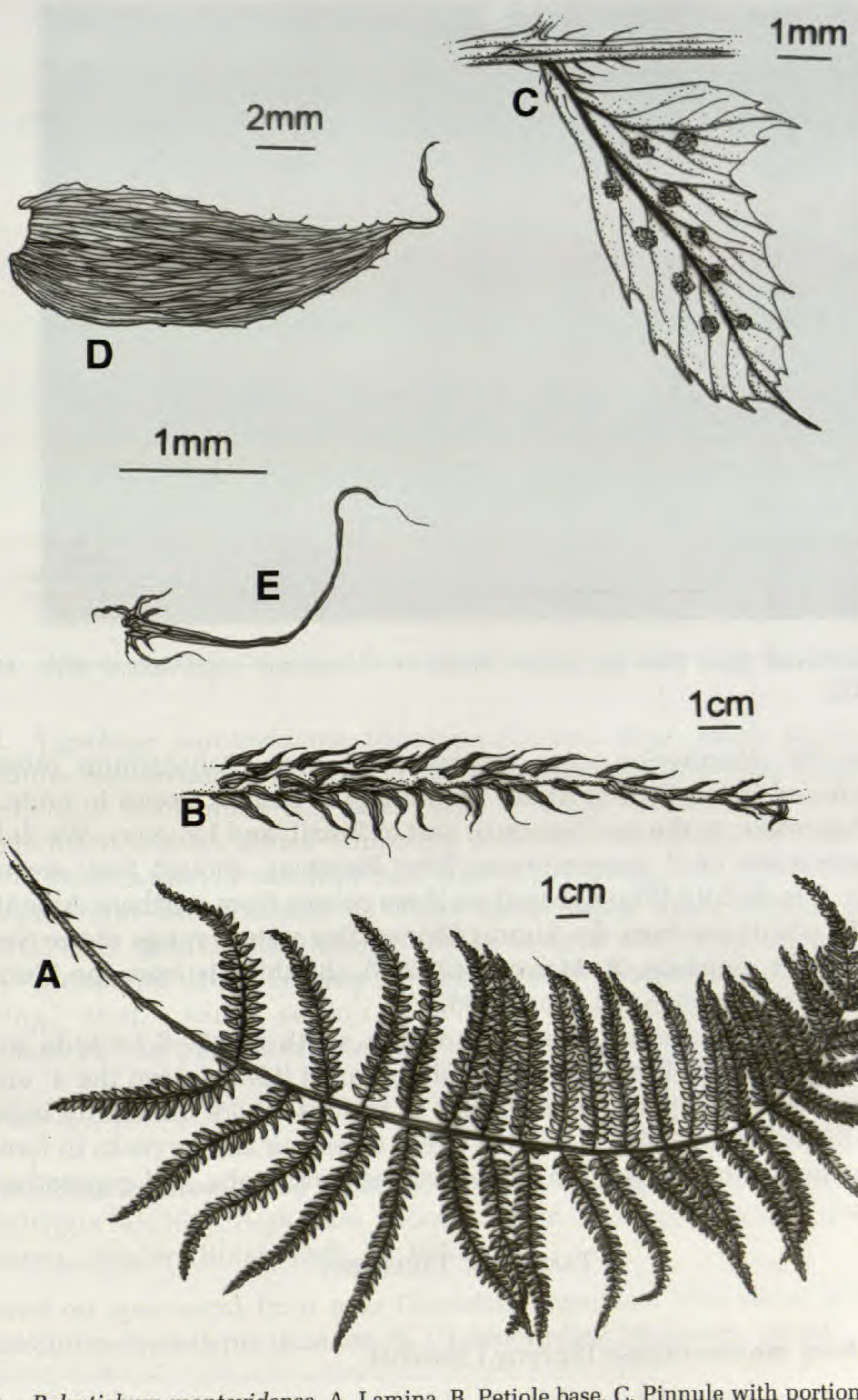

FIG. 2. *Polystichum montevidense*. A. Lamina. B. Petiole base. C. Pinnule with portion of pinna-rachis. D. Basal petiole scale. E. Scale from abaxial surface of pinna-rachis between pinnules. Original material: Uruguay. Pr. Maldonado, *J.P.S. Condack 694* (R).

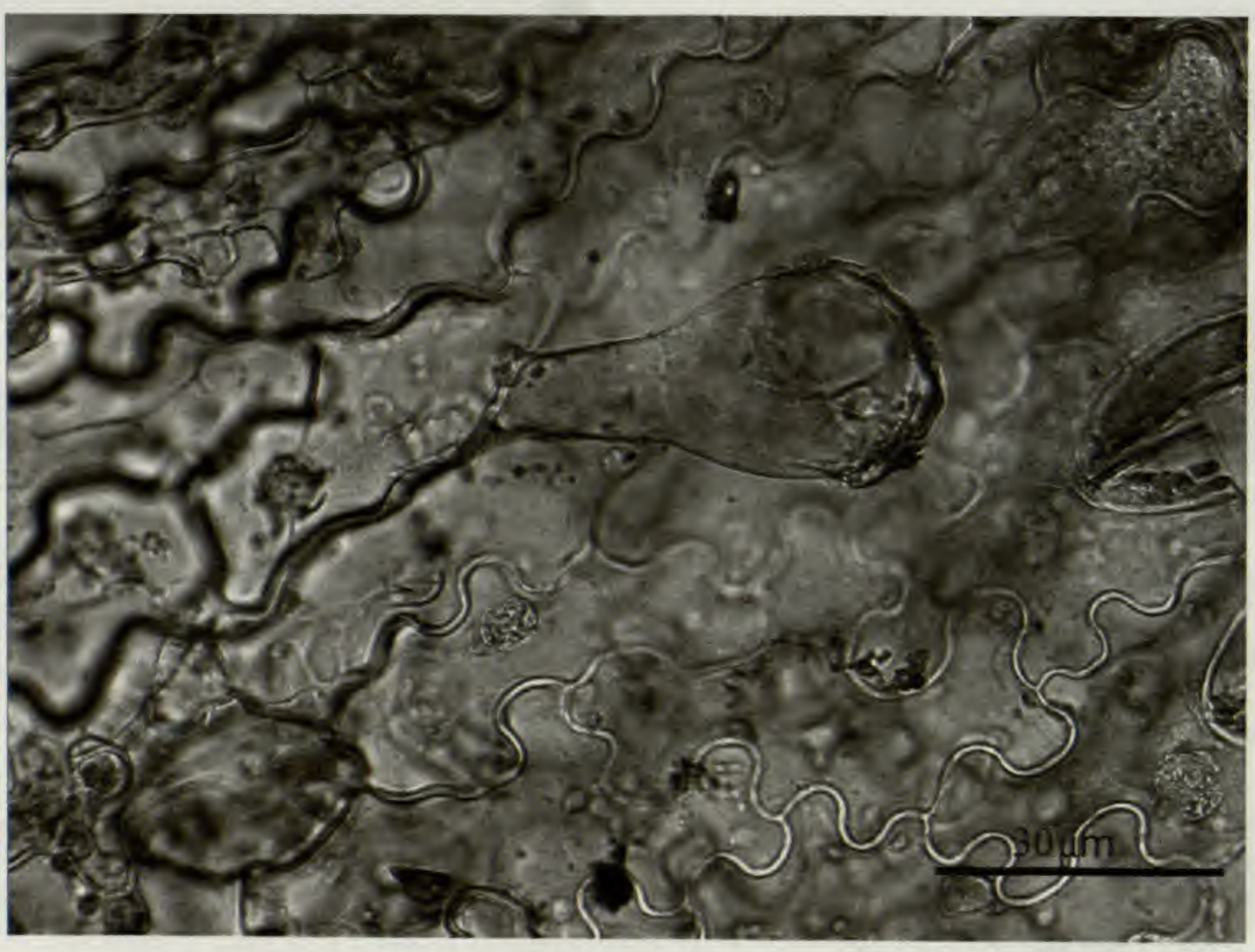

FIG. 3. Epidermal gland from the abaxial lamina of *Polystichum montevidense* (from *Morero 344* [CORD]).

Geographic distribution.—As circumscribed here, *Polystichum montevidense* is found in the Central Andes including the eastern slopes in north and central Argentina, to the southernmost part of Brazil, and Uruguay. We did not locate specimens of *P. montevidense* from Paraguay, though they are to be expected. It is diploid (Fig. 4) based on three counts from northern Argentina: two of the plants are from the Sierras Chicas, the eastern range of the Sierras Pampeanas (Pr. Córdoba, *R. Morero 342, 348*), the third is from the Sierra El Aconquija, Pr. Tucumán, (*R. Morero 352*).

In Brazil the species has been found only south of 30° S latitude in the southernmost state of Rio Grande do Sul. Its range lies between the 4° and 8° minimum-annual-temperature isotherms in regions where the aridity index is near 1.0 (Santibañez, 2004). Habitat is either on soil or among rocks in forested regions or on hilltops in both protected areas under shrubs, and exposed areas.

TAXONOMIC TREATMENT

Polystichum montevidense (Spreng.) Rosenst.

Polypodium montevidense Spreng., Linnaeus, Syst. Veget. ed. 16. 4(1): 59. LECTOTYPE (here designated).—Uruguay, near Montevideo [orig. label: "Brasilia, prope Monte Video"], *Sello d 654* (B 20 0156392; duplicates: B [B 20 0156391, B 20 0156394, B 20 0156395, B 20 0148065], P 01456828). *Phegopteris montevidensis* (Spreng.) Salomon, Nomencl. Gefässkrypt. 276.

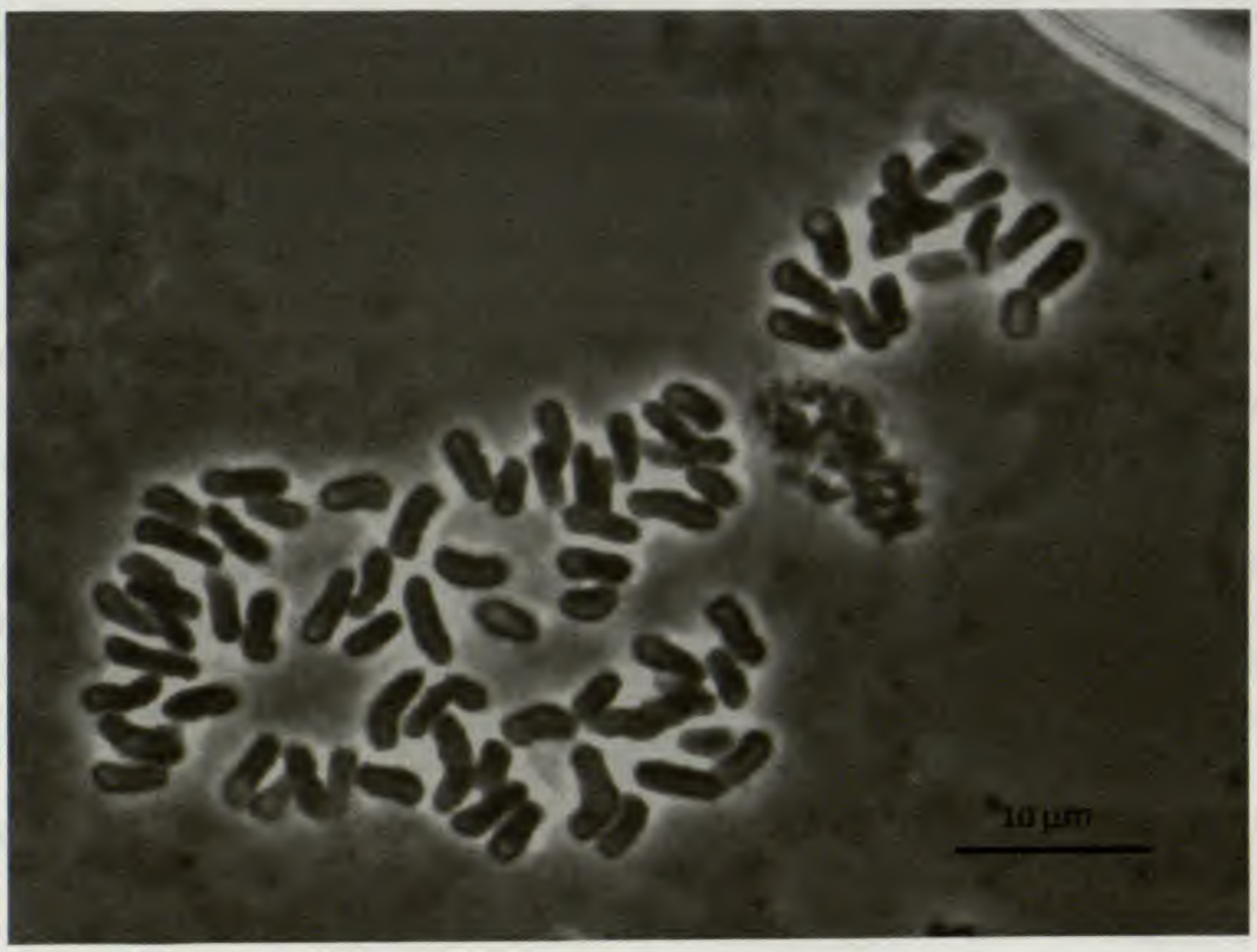

FIG. 4. Mitotic prophase (2n=82) in *P. montevidense* (from a crozier of *Morero 342* [CORD]).

1883. *Aspidium montevidense* (Spreng.) Hieron., Engl. Jahrb. 22:364. 1896. *Aspidium montevidense* (Spreng.) Hieron. in Rosenst., Hedwigia 43:223. 1904. *Polystichum aculeatum* (L.) Roth var. 9, C.Chr. Index Filic. 576. 1905. *Polystichum montevidense* (Spreng.) Rosenst., Hedwigia 46: 111. 1906. *Polystichum montevidense* (Spreng.) Hieron., Hedwigia 46: 356. 1907.

Polystichum sellowianum C. Presl nom. nud., Tent. Pterid. 83.1836. AUTHENTIC MATERIAL, Monte Video, *Sello d 654* (B 20 0156396, with Herb. Mettenius label bearing determination "*Phegopteris montevidensis* (Spreng.) Mett." and a second label with determination "Polystich. sellowianum Pr." in Mettenius's hand.).

Excluded name:

Polystichum montevidense (Spreng.) Rosenst. var. ***squamulosa***, Hieron 1907, Hedwigia 46:356. *Aspidium montevidense* (Spreng.) forma *squamulosa* Hieron., Englers Botan. Jhrb. 22:366.1896.

Based on specimens from near Córdoba, Argentina. This name pertains to *Polystichum pycnolepis* (Kze. ex Kl.) T.Moore (see McHenry, 2012).

DISCUSSION

The correct identity of *Polystichum montevidense,* a name commonly applied to plants across central South America, has been confused for over one hundred years. Even in recent floristic treatments the circumscription of the

species was not informed by the study of authentic material such as the type. Our study is the first to combine extensive fieldwork with analysis of the type collections using modern morphological and molecular methods to clarify the identity of this species.

With the resolution of the major taxonomic problems in Brazilian *Polystichum* (Condack, 2012), comes the realization that *P. montevidense* has been confused most often with a single species, *P. platylepis* (or one of its many synonyms). Plants pertaining to *P. platylepis* were prominent in the collections that Auguste François Marie Glaziou made in the Serra do Mar during the late 1860s. These collections, as they so often served as types for Fée in his *Cryptogames vasculaires du Brésil* (1869; 1872–3), have apparently left botanists confused about the limits of *P. montevidense* and similar species in the Serra do Mar for the last 140 years.

However, the question remains, why is *P. montevidense* so morphologically similar to *P. platylepis*? They share an array of characters including lamina dissection, pinna shape and size, pinnule attachment, and pinnule shape, and petiole scales are similar in shape and hue. They differ in a few key but less salient features, such as petiole-scale cilia development, lamina-indument shape and hue, and spinule development on the pinnules. Two possible explanations merit consideration. First, given the strong tendency to morphological convergence observed in the genus, they may look alike in spite of independent recent histories. Second, however, it is possible that *P. platylepis* is the result of hybridization between *P. montevidense* and another Brazilian species followed by restoration of fertility.

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APPENDIX

Selected specimens examined including all those used in the molecular analysis, which have GenBank accession numbers (marker sequence is *rbcL*, *rps4* gene, *trnLF*, *trnS-rps4*). Herbarium abbreviations throughout follow Index Herbariorum (Theirs, 2012). GAN = GenBank accession number.

Polystichum albomarginatum M. Kessler & A.R. Smith. ECUADOR. **Zamora-Chinchipe:** Bombuscaro, Podocarpus National Park, *M. Lehnert 1257* (VT) (GANs: KC819964; KC819994; KC819949; KC819979).

Polystichum maximum M. Kessler & A.R. Smith. BOLIVIA. **Cochabamba:** Prov. Ayopaya, San Cristobal, 16°39′S, 66°43′W, 2550, *I. Jiménez 1292* (LPB) (GANs: KC819965; KC819995; KC819950; KC819980).

Polystichum montevidense (Spreng.) Rosenst. BOLIVIA. **Santa Cruz**: Caballero, *M. Sundue 844* (VT, NY). **Cochabamba**: Prov. Carrasco, 3.3 km NW of Kayarani, *M. Sundue 621* (VT, NY). **La Paz**: Calle Sorata a Laripata, *M. McHenry 10-95* (VT); Sorata, *M. McHenry 10-100* (VT) (GANs: KC819967; KC819997; KC819952; KC819982). **Tarija**: Arce, Padcaya, *H. Huaylla 1514* (NY). ARGENTINA. **Córdoba**: Dpto. Punilla, Los Gigantes, *R. Morero 342*

(CORD), *344* (CORD) (GANs: KC819968; KC819983; KC819953; KC819998). **Tucumán**: Dpto. Chicligasta. Cuesta del Clavillo, *R. Morero 352* (CORD).

URUGUAY. **Montevideo:** without precise locality, *F. Sello d 654* (B, P); **Maldonado**: Cerro Pan de Azúcar, *Gibert 857* (K); Idem, *A. Lombardo* (BM ex MVM10907); Idem, *A. Lombardo 2568* (MVJB); Idem, *A. Lombardo 4732* (MVJB); Idem, *A. Lombardo 5436* (MVJB); Idem, *J. Arechavaleta 784* (MVFA); Idem, *O. del Puerto et al. 9702* (MVFA); Idem, *E. Marchesi 1379* (MVFA); Idem, *M. Berro 1261* (MVFA); Idem, *J. Condack* & *F. Muñoz* 687 (R) (GANs: KC819966; KC819996; KC819951; KC819981); Idem, *J. Condack 694* (R). **Tacuarembó**: Montes de Tacuarembó, *J. Arechavaleta 4187* (MVM). URUGUAY/BRASIL. **Artigas**: Cuarein (border of Brazil and Uruguay), *M. Berro 5617* (MVFA).

Polystichum nudicaule Rosenst. COLOMBIA. **Boyacá**: Mun. Chisacá-San Pedro de Iquaque, *M. Sundue 1285* (VT) (GANs: KC819969; KC819999; KC819954; KC819984).

Polystichum opacum Rosenst. BRASIL. **Rio Grande do Sul:** Santa Cruz do Sul, *J. Condack & M. Brito 651* (R, VT) (GANs: KC819970; KC820000; KC819955; KC819985).

Polystichum pallidum Gardn. BRASIL. **Rio de Janeiro**: Parque Nacional da Tijuca, *J. Condack 662* (R, VT) (GANs: KC819971; KC820001; KC819956; KC819986).

Polystichum platylepis Fée BRASIL. **Bahia:** Amargosa, *J. Paixão & M. Nascimento 1377* (UEFS). **Espírito Santo:** Castelo, Parque Estadual do Forno Grande, *P. Labiak et al. 4820* (MBML, RB, UPCB). **Mato Grosso:** Vila Bela da Santíssima Trindade, Serra Ricardo Franco, *P. Windisch 1350* (GH, HB, ICN); **Minas Gerais:** Bocaina de Minas, Parque Nacional do Itatiaia, Alto dos Brejos, *J. Condack et al. 328* (RB, NY); **Paraná:** Clevelândia, *G. Hatschbach 22695* (MBM, NY, PACA); **Rio de Janeiro:** Itatiaia, *J. Condack & A. Vasco 636* (R, RBR, VT) (GANs: KC819974; KC820004; KC819959; KC819989). **Rio Grande do Sul:** São José dos Ausentes, *J. Condack & P. Schwartsburd 588* (R, RBR, VT) (GANs: KC819973; KC820003; KC819958; KC819988). **Santa Catarina:** Urubici, Morro da Igreja, *J. Condack & P. Schwartsburd 579* (R, RBR, VT) (GANs: KC819972; KC820002; KC819957; KC819987). **São Paulo:** Bananal, Serra da Bocaina, *A. Brade 15203* (RB).

Polystichum platyphyllum (Willd.) C. Presl. BRASIL. **Goiás:** Caiapônia, Serra do Caiapó, *H. Irwin et al.* 17873 (GH, NY, RB, UB, US).

Polystichum pycnolepis (Kunze ex Klotzsch) T. Moore. COLOMBIA. **Boyacá:** Mun. Villa de Leyva, *M. Sundue 1275* (VT) (GANs: KC819975; KC820005; KC819960; KC819990).

Polystichum rochaleanum Fée. BRASIL. **Rio de Janeiro:** Itatiaia, Pico das Agulhas Negras, *J. Condack & C. Ramos 516* (RB, VT) (GANs: KC819976; KC820006; KC819961; KC819991).

Polystichum rufum M. Kessler & A. R. Smith. BOLIVIA. **F. Tamaya:** PN-ANMI Madidi, *I. Jiménez 1078* (UC) (GANs: KC819977; KC820007; KC819962; KC819992).

Polystichum solomonii M. Kessler & A. R. Smith. BOLIVIA. **Caballero**: 1.5 km down from Empalme, *M. Sundue 775* (VT) (GANs: KC819978; KC820008; KC819963; KC819993).

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Phylogenetic Placement of the Enigmatic Fern Genus *Dracoglossum*

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ABSTRACT.—The phylogenetic position of *Dracoglossum* Christenh. is studied here for the first time using DNA sequence data. Based on a broad sampling of eupolypod ferns and four plastid genes (*atpA*, *atpB*, *rbcL*, *rps4*), we show that *Dracoglossum* does not belong to the genus *Tectaria* Cav., in which it was previously placed, nor is it closely related to that genus or any other member of Tectariaceae. Our results provide strong support to suggest that *Dracoglossum* forms a rather isolated lineage, which is sister to *Lomariopsis* Fée, and that the genus should therefore be placed in Lomariopsidaceae.

KEY WORDS.—Classification, eupolypods, leptosporangiate ferns, Lomariopsidaceae

On rocks in small streams in Neotropical rainforests, a most peculiar fern occurs, which until recently was known as *Tectaria plantaginea* (Jacq.) Maxon. Based primarily on spore characteristics, the species was recently separated from *Tectaria* Cav. and placed in the new genus *Dracoglossum* Christenh. (Christenhusz, 2007). This genus now consists of two species, one – *D. plantaginea* (Jacq.) Christenh. – in Mesoamerica, the lower montane rainforests along the Andes, the Lesser Antilles and Puerto Rico, the other – *D. sinuatum* (Fée) Christenh. – disjunctly in the Guiana shield and coastal Ecuador. The easiest way to distinguish *Dracoglossum* from *Tectaria* Cav. is by its leathery simple leaf blades with apical proliferations. Other simple-bladed species like *Tectaria panamensis* (Hook.) R. M. Tryon & A. F. Tryon and *T. singaporiana* (Wall. ex Hook. & Grev.) Copel. lack these proliferations. Only *Tectaria prolifera* (Hook.) R. M. Tryon & A. F. Tryon has proliferations at the leaf tip, but these leaf-tips are elongated and the leaves are very thin in texture. That species was previously placed in a separate genus *Fadyenia* Hook. based on its strongly dimorphic leaves and unusual lunate indusia, but it is now known that *Fadyenia* is embedded in *Tectaria* (Fig. 1). All these species have echinate spores with wing-like folds, unlike the shallowly ridged spores of *Dracoglossum* (see Tryon & Tryon, 1982 or Christenhusz, 2007).

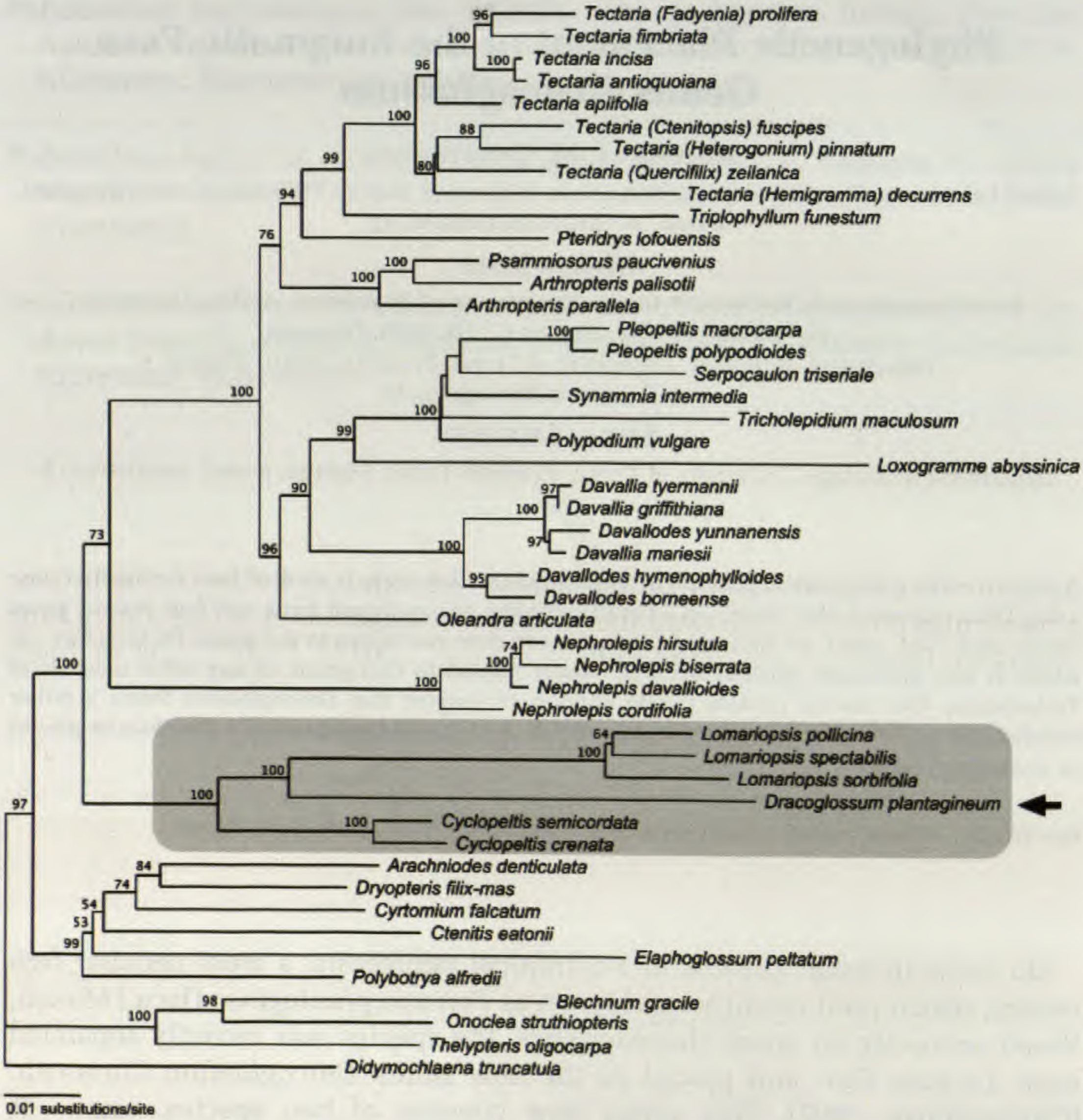

FIG. 1. The best ML tree from the analysis of combined *atpA*, *atpB*, *rbcL* and *rps4* data. Bootstrap support is shown for each node. Lomariopsidaceae is highlighted and the position of *Dracoglossum* within it is indicated.

Classification of ferns has been contentious since the beginnings of fern taxonomy, and even though general morphology remains extremely useful for establishing natural groupings in ferns (e.g. Schneider, 2013; Schneider *et al.*, 2009; Smith, 1995), some genera remained unplaced even after general phylogenetic studies of ferns that included most fern genera, have been carried out (e.g. Hasebe *et al.*, 1995; Manhart, 1995; Pryer *et al.*, 2004; Schuettpelz *et al.*, 2006; Schuettpelz and Pryer, 2007; Lehtonen, 2011). Subsequent placement of enigmatic genera has varied. Some genera have been embedded within larger genera, such as *Hymenophyllopsis* K. I. Goebel that is now known to belong to *Cyathea* Sm. (Korall *et al.*, 2007; Christenhusz, 2009a);

in other cases clades have been divided into separate genera (e.g. Lehtonen *et al.*, 2010; Moran *et al.*, 2010). A number of genera were found to be isolated, resulting in new families needing to be (re-)erected to accommodate them, such as Hypodematiaceae (e.g. Liu *et al.*, 2007; Tsutsumi and Kato, 2006; Schuettpelz and Pryer, 2007), Lonchitidaceae (e.g. Christenhusz, 2009b; Lehtonen *et al.*, 2010; Lehtonen *et al.*, 2012), Diplaziopsidaceae and Rhachidosoraceae (Li *et al.*, 2011; Christenhusz *et al.*, 2011).

In a few cases the ultimate placement was found to be far from where they were originally placed. Psilotaceae were originally placed in the lycopodiophytes (e.g. Reimers, 1954), and were even associated with the earliest fossil land plants, but it has now been shown (Hasebe *et al.*, 1995; Manhart, 1995) that this family forms a clade with Ophioglossaceae. Another good example of the use of molecular phylogeny in the placement of a genus is *Cystodium* J. Sm. in Hook. Originally thought to be a tree fern belonging to Dicksoniaceae (Christensen, 1938; Sen and Mittra, 1966), numerous morphological characters had shown it did not belong there (e.g. Croft, 1986), but nevertheless *Cystodium* remained within Dicksoniaceae (e.g. Kramer & Green, 1990) until a molecular phylogeny showed that it rather belonged among early branching polypods (Korall *et al.*, 2006; Lehtonen *et al.*, 2012). Only a few of these enigmatic genera now remain among ferns.

Most remaining unsampled genera are either impossible to obtain material for, simply because they have not been recently collected, such as *Hypoderris* R.Br. in Wallich, *Oenotrichia* Copel., *Psomiocarpa* C. Presl, *Thysanosoria* Gepp and *Xyropteris* K. U. Kramer, or have simply slipped the attention of phylogenetic treatments. *Dracoglossum* is such an unsampled genus and is one of the few remaining genera that are of unknown affinity. Material from the original collection of Christenhusz (2005) was not available for analysis so a sample of *Dracoglossum plantagineum* (*M. Jones 1018, TUR*) from recent fieldwork on fern communities in central Panama (Jones et al., 2013) was included. A small population of the species was found growing on stones in a shallow stream in the San Lorenzo protected area, ca. 5 km from the Caribbean coast. We analysed this sample to establish the placement of the genus in the general phylogeny of ferns.

Materials and Methods

Taxon and character sampling.—We sampled four chloroplast genes (*atpA*, *atpB*, *rbcL*, *rps4*) from *Dracoglossum* in order to reveal its phylogenetic position. Total genomic DNA was extracted with an E.Z.N.A. SP Plant DNA Kit (Omega Bio-tek, Doraville, GA). PCR reactions were performed using PureTaq RTG PCR beads (Amersham Biosciences, Piscataway, NJ, USA) and primers ATPF412F and TRNR46F for *atpA* (Schuettpelz *et al.*, 2006), ATPF412F and TRNR46F for *atpB* (Schuettpelz *et al.*, 2006), ESRBCL1F and ESRBCL1361R for *rbcL* (Korall *et al.*, 2006), and trnS^{GGA} and rps4.5′ for *rps4* (Shaw *et al.*, 2005). Purification of PCR reactions and DNA sequencing were carried out under BigDye^{TM} terminator cycling conditions by Macrogen Inc., Seoul, South Korea/

Amsterdam, the Netherlands (www.macrogen.com), using the same primers that were used for amplification. The resultant sequences were deposited in GenBank. Sequence data of several eupolypod I lineages were downloaded from GenBank for use in the analysis, as well as a few eupolypod II species for outgroup comparison (see Appendix 1 for GenBank accession numbers).

Phylogenetic inference.—We performed a multiple sequence alignment with default parameters in Mafft 7.029b (Katoh and Standley, 2013) and pruned the incomplete 5′ and 3′ ends of each partition based on visual inspection of the alignments. Sequence alignment was straightforward, as no gaps were required. Data matrices were concatenated with SequenceMatrix 1.7.8 (Vaidya *et al.*, 2011) and the resulting supermatrix was analysed under the Maximum Likelihood criterion using parallel Pthreads-version of the computer program RAxML 7.3.0 (Stamakis, 2006; Ott *et al.*, 2007). Free model parameters were estimated by RAxML under the GTR+Γ model. We combined bootstrap support (BS) calculation (1000 replicates) with a full ML search using the commands: *./raxmlHPC-PTHREADS-SSE3 -T 16 -f a -x 80509 -p 37023 -# 1000 -m GTRGAMMA -s totaldata.raxml -n Dracoglossum*. We also used a similar search to analyse each gene separately in order to evaluate possible conflict among the datasets.

Results

Separately analysed genes did not show incongruent topologies supported by a BS $\geq$ 70%, with the exception of *Nephrolepis davallioides* Kunze. This species was resolved as the sister of *N. hirsutula* (G. Forst.) C. Presl by *rbcL* and *atpB* genes, but as the sister of the *N. hirsutula–N. biserrata* (Sw.) Schott clade by the *rps4* gene and in the combined analysis. This conflict is considered irrelevant here, and the phylogenetic relationships presented are based on analyses of the combined data set (Fig. 1). *Dracoglossum*, the focus of our study, is resolved as part of the eupolypod I fern clade (e.g. Smith *et al.*, 2006) with high support (Bootstrap support 100%; Fig. 1). Within this clade there is weak support (73%) for the placement of *Nephrolepis* Schott as sister to the crown group comprising of Oleandraceae, Davalliaceae, Polypodiaceae and Tectariaceae *sensu stricto*. Sister to this assembly is a clade in which *Dracoglossum* is placed with strong support with *Lomariopsis* Fée (100%), a clade which in turn is sister to *Cyclopeltis* J.Sm. with strong support (100%). Within this clade *Dracoglossum* appears rather isolated on a relatively long branch.

Discussion

Because of the placement of *Dracoglossum* as sister to *Lomariopsis*, a comparison of the two genera is necessary to assess morphological similarities and adjust the circumscription of Lomariopsidaceae. Plants of Lomariopsidaceae are terrestrial, epilithic or hemi-epiphytic, and have creeping rhizomes, often scrambling over rocks (all genera) or climbing up trees (*Lomariopsis*), but

sometimes the rhizomes are stout and short (some *Cyclopeltis*). Leaves monomorphic (*Cyclopeltis*, *Dracoglossum*) or dimorphic (*Lomariopsis*). Petioles have round vascular bundles arranged in a U-shape. Blades are simple and proliferous at the apex (*Dracoglossum*) or once pinnate and not proliferous (*Cyclopeltis*, *Lomariopsis*). Pinnae articulate to the rachis (*Cyclopeltis*, *Lomariopsis*) when present. Veins free, parallel, simple to forked several times (*Cyclopeltis*, *Lomariopsis*) or meniscioid-reticulate with included veinlets (*Dracoglossum*). Sori discrete, orbicular, with peltate indusia or exindusiate, placed in 1–4 rows on each side of costa (*Cyclopeltis*, *Dracoglossum*) or sporangia acrostichoid, covering entire lamina of the contracted fertile leaf (*Lomariopsis*). Spores bilateral, monolete, variously winged or ornamented. The chromosome numbers are also different, *Lomariopsis* and *Cyclopeltis* having x=41, whereas *Dracoglossum* has x=40 (Walker, 1985).

Conclusion.—On the basis of our findings we conclude that the fern family Lomariosidaceae, should include four genera: *Cyclopeltis*, *Dracoglossum*, *Lomariopsis* and *Thysanosoria*. The genus *Nephrolepis*, sometimes included in Lomariopsidaceae (e.g. Smith *et al.*, 2006), is better placed in its own family because its affinities are still unclear and may not necessarily lie within Lomariopsidaceae. *Thysanosoria*, which still lacks molecular data, because it is only known from a few old herbarium specimens, appears to have close affinity with *Lomariopsis* and may need to be merged with that genus. Synapomorphies for the redefined Lomariopsidaceae are: creeping rhizomes; dark rhizome scales; petioles with vascular bundles arranged in a U-shape; leaves simple to once pinnate, the pinnae articulate to the rachis when present; spores bilateral and monolete. These characters are not very distinctive, and with the inclusion of *Dracoglossum*, the family becomes difficult to define, a rather similar situation as in the circumscription of Hypodematiaceae, which also includes three widely different genera. In spite of all the differences, *Dracoglossum* shares characters with both *Lomariopsis* and *Cyclopeltis*, and thus its placement among them is not completely unwarranted.

An affinity of *Dracoglossum* with the Caribbean genus *Hypoderris* has also been suggested (Christenhusz, 2007), but that genus has not yet been studied, although it will be included in a current global molecular study of Tectariaceae.

Avoiding families with few synapomorphies, an alternative classification for this group would be a two-family concept for eupolypods (resp. Polypodiaceae for eupolypods I and Aspleniaceae for eupolypods II). The numerous small segregate families (often consisting of one or a few genera) were proposed simply to maintain established families. Such a radical classification may be worth considering in future classifications, especially if suitable synapomorphies for these enlarged families can be found to distinguish readily between the two (although the inclusion of *Thelypteris* Adans. and relatives in Aspleniaceae will make this difficult). For stability purposes, however, the classification of Smith *et al.* (2006) and Christenhusz *et al.* (2011) are adopted

here and thus *Dracoglossum* is placed in Lomariopsidaceae, not in an expanded Polypodiaceae.

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APPENDIX 1.

GenBank accessions used in this study (taxa, *atpA*, *atpB*, *rbcL*, *rps4*).

Arachniodes denticulata, EF463664, EF463380, AF537223, –; *Arthropteris palisotii*, JF304014, –, AB575230, –; *Arthropteris parallela*, EF463862, EF463522, EF463266, –; *Blechnum gracile*, EF463615, EF463351, EF463158, AF313606; *Ctenitis eatonii*, JF304011, EF450515, U05614, EF540713; *Cyclopeltis crenata*, JF304016, EF450534, DQ054517, EF540718; *Cyclopeltis semicordata*, JF832107, EF463480, EF463234, –; *Cyrtomium falcatum*, EF463671, EF463387, AF537226, –; *Davallia griffithiana*, EF463649, EF463371, EF463165, –; *Davallia mariesii*, –, AB212706, U05617, JX103759; *Davallia tyermannii*, –, JX103677, JX103719, JX103761; *Davallia yunnanensis*, –, JX103676, JX103718, JX103760; *Davallodes borneense*, EF463650, AB212694, AB212694, –; *Davallodes hymenophylloides*, EF463648, AB212689,

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American Fern Journal 103(2):139–140 (2013)

Shorter Notes

Predation of Bracken Fern (*Pteridium aquilinum* (L.) Kuhn var. *arachnoideum* (Kaulf.) Brade) in Southern Brazil by Moths of the Genus *Erupa*.—*Pteridium aquilinum* (L.) Kuhn (*sensu lato*), the bracken fern, is one of the most important toxic plants impacting cattle and sheep as well as being a highly invasive species in pastures and agricultural fields worldwide. The diverse problems caused by bracken have lead to the search for methods to control its establishment and proliferation, including mechanical, chemical and biological approaches. The search for biological control organisms lead to diverse reports of herbivorous insects found on bracken (e.g. Balick *et al.*, Oecologia, 35, 55–89. 1978; and also Mehltreter, Pp 220–257, *in* Mehltreter, Walker & Sharpe, Fern Ecology, Cambridge University Press, 2012).

This note reports the predation of *Pteridium aquilinum* (L.) Kuhn var. *arachnoideum* (Kaulf.) Brade [*P. esculentum* subsp. *arachnoideum* (Kaulf.) J.A.Thomson, according Thomson, Telopea 14:43–48. 2012)] by larvae of a moth of the genus *Erupa*, which develop inside the petiole bases in populations occuring in Southern Brazil.

Rhizomes with at least some leaf bases presenting signs of predation in the form of isolated perforations were collected from a population growing next to an artificial lake at the campus of the Universidade do Vale do Rio dos Sinos, São Leopoldo (ca. 60 m alt.), State of Rio Grande do Sul, Brazil, in May 2003 (voucher at herbarium PACA, leg. G. Fausti). Longitudinal sections showed from larvae to pupae developing in cavities within the petioles. Generally there was a single infesting organism per leaf base, but up to nine were observed in one case. The larvae become pupae within the plant tissue, surrounded by their cocoon. After the pupal phase the adult moth emerges through a perforation in the cortical plant tissue. The presence of cavities produced by moths was reported for *Acrostichum danaefolium* Langsd. & Fisch. (Mehltreter *et al.*, Amer. Fern J. 93:48–54. 2003) occupied by ants. In the present case the rhizomes with leaf bases were removed from the field and taken into culture, and no secondary occupation of the cavities was observed. The samples presented diverse stages of development, indicating that the propagation of the moth is probably continuous.

Twenty five rhizome apexes with three petiole bases presenting signs of infestation were randomly collected and kept in boxes (five samples per box) with humid soil, initially closed at the top with netting (1.5 mm squares). After the first week the material was transferd to more permanent boxes, closed by a top lid with a circular orifice on which a clear plastic cup was attached in order to allow easier capture of any light attracted insect emerging from the plant material. The boxes were kept at room temperature under indirect daylight. It was not possible to establish the developmental stages of the infesting organisms at the time of collection, but after two and up to five months three moths emerged.

The moths were identified to genus with the kind assistence of Mr. Alfred Moser (São Leopoldo, Brazil), and confirmed by Dr. Victor O. Becker (Federal University of Brasilia, Brazil, voucher specimen) as Lepidoptera, Crambidae, genus *Erupa* Walker, *Erupa* cf. *bilineatella* (Walker, 1866). In one of the boxes the emergence a parasitoid wasp was also observed. According to Dr. Angélica Pentado-Dias (Federal University of São Carlos – UFSCAR, Brazil) this vespid Hymenoptera probably is a parasite of the *Erupa* larvae (specimen at UFSCAR).

Between July 2004 to June 2005, three additional bracken stands in the northeastern region of the State of Rio Grande do Sul (Sapiranga, ca. 40 m alt, ca. 20 km from São Leopoldo; Canela, ca. 900 m alt, ca 55 km; São Francisco de Paula, 950 m alt., ca. 64 km, forming a poligonal area with ca 650 km^2) were visited. In a total sample of 78 leaves, 32.05% were infested, all presenting similar morphological characters as to the damage on the plant material, suggesting a single infesting species and documenting that the infestation was not restricted to the first population. Aside from the damage on the petiole bases, no differences were observed on the infested leaves when compared to those without larvae. Images of the moth and larvae can be provided by the second author.

The authors thank the cited entomologists for the identifications and the reviewers for helpful suggestions.—Gabriela Fausti Landoni, (Via Sant'Antonio 66, 21040 Cislago – VA, Italy), and Paulo G. Windisch, (Brazilian Research Council – CNPq grant), PPG-Botânica, Univ. Federal do Rio Grande do Sul, 91540-970 Porto Alegre-RS, Brazil, e-mail: pteridos@gmail.com.br.

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Molecular Phylogenetic Relationships of *Cibotium* and Origin of the Hawaiian Endemics

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ABSTRACT.—The tree fern genus *Cibotium* comprises nine species distributed in tropical regions of Asia, Mesoamerica, and the Hawaiian Islands. The four Hawaiian species are endemic to the Hawaiian Islands. The goals of this paper were to determine the relationships among the *Cibotium* species, determine whether the Hawaiian species are monophyletic, and infer the dispersal pathway likely responsible for delivering an ancestral *Cibotium* species to the Hawaiian Islands. Molecular phylogenetic analyses based on four coding and five non-coding plastid DNA sequences supported Hawaiian *Cibotium* as monophyletic, suggesting a single colonization of the Hawaiian Islands. Hawaiian *Cibotium* are most closely related to species in Mesoamerica. If the ancestor of Hawaiian *Cibotium* dispersed to the Hawaiian Islands via wind dispersed spores, our analyses suggest the trade winds or storms delivered spores from Mesoamerica or the Hawaiian Islands were colonized first by a species from Asia, followed by subsequent dispersal to Mesoamerica from Hawai'i. Our analyses do not allow us to favor one hypothesis over the other.

KEY WORDS.—Hawai'i, historical biogeography, *Cibotium*, spore dispersal, molecular phylogenetics, tree ferns

The current high islands of the Hawaiian Islands are located approximately 4000 km from the nearest continent, North America, and 1600 km from the nearest archipelago, the Marquesas Islands of Polynesia. The Hawaiian Island chain is about 80 million years old and was produced in a conveyer-belt-like manner by the Hawaiian hotspot beneath the Pacific Ocean. Islands are removed from the hotspot as the Pacific tectonic plate moves to the northwest (e.g., Clague and Dalrymple, 1987). Once removed from the hotspot, the islands eventually erode to or below sea level. Geological evidence suggests that the islands' separation from the mainland has been relatively constant (Carson and Clague, 1995) and their extreme isolation has been a barrier to colonization by terrestrial organisms. Because the islands are volcanic in origin and are so isolated, colonizing species must have dispersed to the islands via wind or water.

The Hawaiian Islands have the highest levels of species endemism of any regional flora in the world (Wagner *et al.*, 1999), which can be partially explained by the continual opening of new habitats accompanied by dispersal of organisms throughout the developing islands. Of the 136 species of ferns and lycophytes native to the Hawaiian Islands, 67% are endemic to the archipelago (Vernon, 2011). Ferns and lycophytes comprise about 12% of the native vascular-plant species of the Hawaiian Islands (Warren L. Wagner, 2011 pers. comm. and http://botany.si.edu/pacificislandbiodiversity/hawaiianflora/), although they constitute only about 3% of the vascular-plant flora of the world.

During the past 80 Ma the size of islands produced by the activity of the Pacific hotspot has varied (Price and Clague, 2002). Price and Clague (2002) indicate that there was a lull in island formation from 33 Ma to 23 Ma ago. During this time, the lack of available habitats would have resulted in extinction of most or all Hawaiian terrestrial biota such that there was essentially a renewal of terrestrial life beginning 23 Ma ago. Prior to 18 Ma ago, there were few islands with peaks over 1000 m, but between approximately 18 Ma and 11 Ma ago there were numerous larger peaks over 1000 m, including some greater than 2000 m. However, due to subsequent erosion of those larger peaks, the models produced by Price and Clague (2002) indicate there were few islands higher than 1000 m during the 5–6 Myr preceding the formation of Kauai 5.2 Ma ago. Because of this, Price and Clague (2002) suggested that montane taxa, including most of the ferns, probably arrived from outside the Hawaiian archipelago or evolved after the formation of Kauai approximately 5.2 Ma ago because appropriate mid- and high-elevation montane habitats did not exist on islands older than Kauai by the time Kauai was high enough to support these habitats.

Geiger *et al.* (2007; and references therein) identified four potential weather- or climate-based pathways for spore dispersal that could explain the biogeographic origins of native Hawaiian fern lineages. They suggested the northern subtropical jetstream could carry spores to the Hawaiian Islands from areas of the Indo-Pacific; spores could be moved from Central and North America via the trade winds; spores could be carried by storms from southern Mesoamerica; or spores could be transported from the Southern Hemisphere (S. America or the South Pacific) northward across the equator by a combination of mechanisms involving a seasonal southern shift of the Intertropical Convergence Zone (ITCZ), movement of air via Hadley Cells, and the trade winds (see Wright *et al.*, 2001). Reviews of studies by Geiger *et al.* (2007) that included Hawaiian endemic species (*Dryopteris* (Geiger and Ranker, 2005), *Hymenophyllum* (Ebihara *et al.*, 2004; Hennequin *et al.*, 2006), *Polystichum* (Driscoll and Barrington, 2007), *Adenophorus, Grammitis*, and *Stenogrammitis* (Ranker *et al.*, 2003, 2004; *Stenogrammitis* treated as *Lellingeria* in the works cited)) found evidence for each of the four described weather/climate-based dispersal mechanisms. In the current study, using molecular phylogenetics, we evaluated the biogeographical history of the

genus *Cibotium*, which includes four Hawaiian endemic species, in light of the four weather/climate-based dispersal mechanisms.

The monophyletic tree fern genus *Cibotium* (Cibotiaceae) (Korall *et al.*, 2006; Smith *et al.*, 2006) comprises nine species (see Table 1) (Kramer 1990c; Palmer, 1994). In the Hawaiian Islands it is represented by four endemic species, in Asia by three species, and in Mesoamerica by two species (Kramer, 1990c; Palmer, 1994). The genus is characterized by large rhizomes, leaves 2–4 m in length, hairy petiole bases, and a solenostele or dictyostele (Smith *et al.*, 2006). The spores of *Cibotium* show varying degrees of exine distal ridge development (Gastony, 1982). The spores of Asian species have the greatest degree of distal and equatorial ridge development, while those of the Hawaiian species intergrade with those from the Mesoamerican species, which have the least developed ridges (Gastony, 1982).

Fosberg (1948), who hypothesized geographic origins for the original colonizing ancestors of native Hawaiian ferns and lycophytes, hypothesized one colonizing ancestral species for Hawaiian *Cibotium* that probably originated in the Indo-Pacific, but he could not rule out the possibility of an American origin. The general goal of this research was to test Fosberg's (1948) hypothesis using molecular phylogenetic analyses, and to specifically: 1) determine if the Hawaiian species of *Cibotium* are monophyletic; and 2) determine the relationships among the nine species of *Cibotium*, elucidating the biogeographic origin(s) of the Hawaiian species, and thus identifying which climate-based dispersal pathway (Geiger *et al.*, 2007) most likely explains how the ancestor(s) of the Hawaiian species was delivered to the Hawaiian Islands.

Materials and Methods

Sampling and molecular methods.—All nine species of *Cibotium* were sampled in this study, as well as three outgroup species chosen based on relationships revealed by Korall *et al.* (2006). The three outgroup taxa, *Calochlaena villosa*, *Dicksonia antarctica*, and *Lophosoria quadripinnata*, belong to the Dicksoniaceae as circumscribed by Smith et al. (2006), a well-supported group closely related to *Cibotium*. Representatives of the genus *Metaxya* were not included as outgroups due to their unclear phylogenetic position (Korall *et al.*, 2006). Taxa included in this study, voucher information, and database accession numbers are listed in Table 1.

Total DNA was extracted using the DNeasy plant mini kit from Qiagen (Valencia, CA, USA). Four coding (*atpA*, *atpB*, *rbcL*, *rps4*) and five non-coding (*rbcL–atpB*, *rbcL–accD*, *rps4–trnS*, *trnG–R*, and *trnL–F*) plastid DNA regions were PCR amplified and sequenced. When possible, sequences accessible through GenBank were utilized. In Geiger's lab, *rbcL* and *trnL–F* were amplified as in Geiger and Ranker (2005), amplification of *atpB* followed the methods described in Ranker *et al.* (2003), amplification of *trnG-R* followed the methods of Korall *et al.* (2007), and the *rps4–trnS* IGS was amplified following

TABLE 1. Species list, collection, and voucher information, and database accession numbers.

Taxa examined in study					
Species	Family	Voucher	Fern DNA DB#	*atpA*	*atpB*
Ingroup					
Cibotium arachnoideum (C.Chr.) Holttum	Cibotiaceae	Ranker 2010 (COLO; TAIF)	NA	JX485654 [3]	JX485655 [3]
Cibotium barometz (L.) J.Sm.	Cibotiaceae	Larsen et al. 46302 (AAU)	2277	AM176429[1]	AM176589[1]
Cibotium chamissoi Kaulf.	Cibotiaceae	Morter 2 (E)	2486	AM176430[1]	AM176590[1]
Cibotium cumingii Kunze	Cibotiaceae	Ranker 2070 (COLO; TAIF)	NA	NA	JX485656 [3]
Cibotium glaucum (Sm.) Hook. & Arn.	Cibotiaceae	Wolf 266 (Strybing Herbarium)	132	AM176431[1]	AM176591[1]
Cibotium menziesii Hook.	Cibotiaceae	Ranker 1996 (COLO; BISH)	NA	NA	JX485657 [3]
Cibotium nealiae Degen.	Cibotiaceae	Fagerlind & Skottsberg 6494 (S)	2253	AM176432[1]	AM176592[1]
Cibotium regale Versh. & Lem.	Cibotiaceae	Oxford Botanic Garden	NA	NA	JX485658 [3]
Cibotium regale Versh. & Lem.	Cibotiaceae	Royal Botanic Garden 19991656	NA	NA	–
Cibotium schiedei Schlecht. & Cham.	Cibotiaceae	Morter 4 (E)	2485	AM176433[1]	AM176593 [1]
Outgroup					
Calochlaena villosa (C. Chr.) M. D. Turner & R. A. White	Dicksoniaceae	Woodhaus (AAU)	2254	AM176428[1]	AM176588[1]
Dicksonia antarctica Labill.	Dicksoniaceae	Wolf 276 (UTC)	134	AM176442[1]	**U93829**
Lophosoria quadripinnata (J. F. Gmel.) C. Chr. (Type sp.)	Dicksoniaceae	Grantham 006-92 (UC)	424	AM176450[1]	AM176609[1]

A dash (—) indicates that data are available for this taxon from a different voucher
XXX = new to this study, accession numbers inserted later
NA = Data not available for this taxon
[1] from Korall et al., 2006
[2] from Korall et al., 2007
[3] sequenced by JMOG
[4] sequenced by PK
Bold – from other studies

Li and Lu (2006). PCR products were purified with ExoSAP-IT (USD Corp.) and sequenced by Macrogen Inc. in South Korea. Sequences produced by Korall were amplified and sequenced using the primers and methods described in Korall *et al.* (2006, *atpA*, *atpB*, *rbcL*, *rps4*, and *rps4–trnS* IGS

TABLE 1. Extended.

rbcL	*rps4*	*rbcL–accD*	*rbcL– atpB*	*trnGR*	*trnLF*	*rps4–trnS* IGS
JX485690 [3]	NA	NA	NA	NA	NA	NA
AM177328[1]	AM176485[1]	JX485694 [4]	NA	JX485674 [4]	JX485682 [4]	JX485663[4]
AM177329[1]	AM176486[1]	JX485695 [4]	JX485659 [4]	JX485675 [4]	JX485683 [4]	JX485664[4]
JX485691 [3]	NA	NA	NA	JX485676 [3]	JX485684 [3]	JX485665[3]
U05913	AM176487[1]	JX485696 [4]	JX485660 [4]	JX485677 [4]	JX485685 [4]	JX485666[4]
JX485692 [3]	NA	NA	NA	JX485678 [3]	JX485686 [3]	JX485667[3]
AM177330[1]	AM176488[1]	JX485697 [4]	JX485661 [4]	JX485679 [4]	JX485687 [4]	JX485668[4]
–	NA	NA	NA	JX485680 [3]	JX485688 [3]	JX485669[3]
JX485693 [3]	NA	NA	NA	–	–	–
AM177331[1]	AM176489[1]	JX485698 [4]	JX485662 [4]	JX485681 [4]	JX485689 [4]	JX485670[4]
AM177327[1]	AM176484[1]	AM410497[2]	AM410290[2]	AM410426[2]	AM410354[2]	JX485672[4]
U05919	**AF313596**	AM410498[2]	AM410291[2]	AM410427[2]	AM410355[2]	JX485671[4]
AF101303	AM176502[1]	AM410505[2]	AM410298[2]	AM410434[2]	AM410361[2]	JX485673[4]

(amplified and sequenced within *rps4*)) and Korall *et al.* (2007, *rbcL–accD*, *rbcL–atpB*, *trnG–R*, and *trnL–F*).

Sequence editing and alignment.—Sequence fragments were assembled and edited using Sequencher version 4.2.2 (Gene Codes, Ann Arbor, Michigan, USA). The edited consensus sequences were aligned manually using MacClade version 4.07b13 (Maddison and Maddison, 2005). Ambiguously aligned regions (due to insertions or deletions) in the noncoding regions (*rbcL–accD*, *rbcL–atpB*, *trnG–R*, *trnL–F*, and *rps4–trnS* IGS) were excluded from the analyses. No "gap coding" was performed.

TABLE 2. Number of characters, summary of nucleotide substitution models used in Bayesian (B/MCMC) analysis, and sequence analysis for the nine ingroup and three outgroup taxa.

Data set	No. char	B/MCMC substitution model
atpA	1514	HKY+I
atpB	1482	HKY
rbcL	1309	HKY+G
rbcL-accd	1452	GTR+I
rbcL-atpB	640	HKY+G
rps4	603	HKY
rps4-trnS	466	HKY+G
IGS		
trnGR	1050	HKY+G
trnLF	1065	HKY+I
Combined		--------
Aligned	9581	
Excluded	105	
Analyzed	9476	

Phylogenetic analyses.—We tested for incongruence among the nine single-region data sets by analyzing the data sets separately using both a Bayesian Markov chain Monte Carlo approach (B/MCMC) and equally weighted Maximum Parsimony (MP) with settings as described below for the combined data set. We compared the resultant topologies and incongruence supported by a Bayesian posterior probability of $\geq 70\%$ was considered a conflict. For each analytical method we compared all nine resulting topologies and no conflicts were found in the ingroup. The nine single-region data sets were combined into a single data set and analyzed using a Bayesian Markov chain Monte Carlo approach (B/MCMC) and equally weighted Maximum Parsimony (MP). B/MCMC analyses were performed with MrBayes 3.1.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), using a single partition for each region (i.e., with nine partitions). The most appropriate nucleotide substitution models for each of the regions were determined using the Perl script MrAIC version 1.4.4 (Nylander, 2004) in combination with PHYML version 2.4.4 (Guindon and Gascuel, 2003). The choice of model was based on the corrected Akaike information criterion (AICc) (see Table 2 for a summary of models used). The analysis was run for five million generations, on four parallel chains, with the temperature parameter set to 0.2. Four independent analyses of each region were run simultaneously. To determine whether parameters had converged, the values sampled for different parameters were examined using the program Tracer v. 1.2.1 (Rambaut and Drummond, 2005). We also examined the standard deviation of the split frequencies among the independent runs as calculated by MrBayes. For each analysis, every 1000^{th} tree was sampled, and burn-in was conservatively set to one million generations. A majority-rule consensus tree was calculated based on the pool of trees resulting from the four independent analyses (except those trees discarded as burn-in).

The MP analyses were performed with PAUP* version 4.0b10 (Swofford, 2002) and included a heuristic search for the most parsimonious trees with 1000 random-sequence-addition replicates with MulTrees activated and tree-bisection-reconnection (TBR) branch swapping. Support for nodes was calculated by bootstrap analysis with 5000 bootstrap replicates with 10 random-sequence-addition replicates each.

All trees were rooted with the three outgroup species.

Results

The number of characters included in the analyses and the nucleotide substitution model used in B/MCMC analyses are summarized in Table 2. For the combined MP analysis 407 (4.3%) characters were parsimony informative. The heuristic MP search resulted in three most parsimonious trees, each with a length of 977 steps in one island.

Our results indicated that, based on plastid data alone, *Cibotium* was strongly supported as monophyletic and most relationships among the *Cibotium* species were strongly supported (posterior probability, PP = 1.00) according to the B/MCMC analysis, and they were moderately (bootstrap percentage, BP = 70–89%) to strongly supported by the MP analysis (BP ≥ 90%) (Fig. 1). Few indels were found, and these were either autapomorphies or synapomorphies for clades already well supported. Most indels supported the monophyly of the ingroup. Only two indels were synapomorphies of subclades within the ingroup: a five-base indel joining *Cibotium regale* and *C. schiedei* and a seven-base indel shared by *C. chamissoi* and *C. menziesii*.

Within *Cibotium*, there were three strongly supported clades that correspond to the geographic distributions of the included species: the Asian clade, with three species, was sister to a clade with the four Hawaiian species in one subclade, and the two Mesoamerican species in the other. Within the Asian group, *C. arachnoideum* was well supported by B/MCMC analysis and moderately supported by MP analysis as sister to the pair *C. barometz* and *C. cumingii*. The sister relationship between the Hawaiian species and those from Mesoamerica was strongly supported by both analyses. Within the Hawaiian clade, *C. chamissoi* and *C. menziesii* were strongly and moderately supported as sister species by B/MCMC and MP analyses, respectively. However, the relationships among those two taxa and *C. nealiae* and *C. glaucum* were unresolved.

Discussion

The goals of this study were to determine if the Hawaiian species of *Cibotium* are monophyletic and to determine the relationships among species of *Cibotium* to infer the biogeographical history of the Hawaiian species. Using five coding and four non-coding plastid DNA loci, this study has resulted in a relatively well-resolved molecular phylogeny for *Cibotium*. Within *Cibotium*, three clades were resolved, each of which corresponds to the geographical

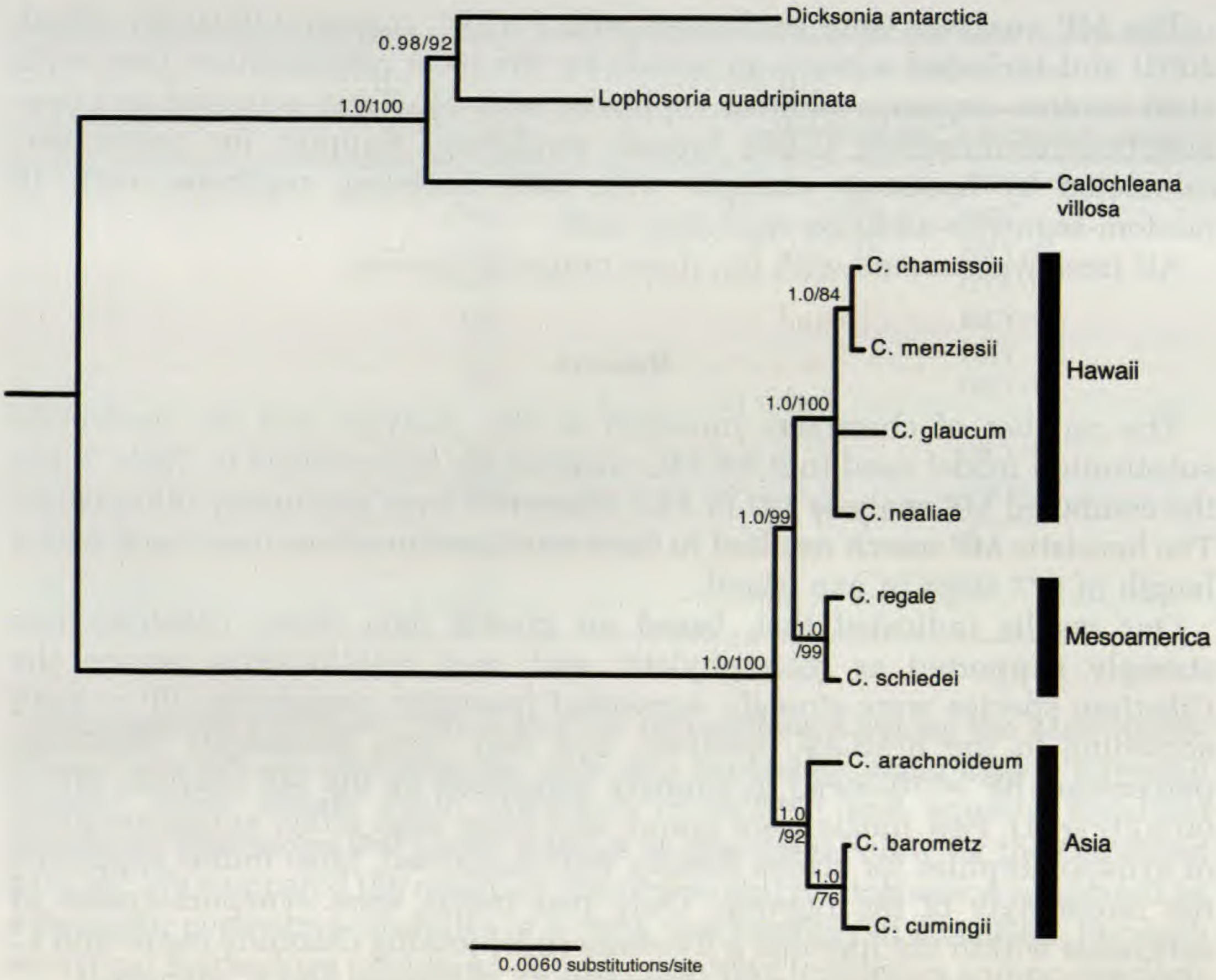

FIG. 1. The 50% majority-rule consensus tree resulting from Bayesian (B/MCMC) analyses of the combined data set (four coding (*atpA*, *atpB*, *rbcL*, *rps4*) and five non-coding (*rbcL–atpB*, *rbcL–accD*, *rps4–trnS* IGS, *trn-GR*, and *trnL-F*). Support values from Bayesian analysis (posterior probabilities (PP)) are in front of the slash, and support values from maximum parsimony (MP) are behind the slash.

distributions of the component species. We do interpret the results with caution, however, as the phylogeny is solely based on plastid data.

The four endemic Hawaiian *Cibotium* species comprise a well-supported clade (Fig. 1). Based on this, we infer there was a single *Cibotium* ancestral species that colonized the Hawaiian Islands, followed by subsequent diversification of the species. The Hawaiian clade is strongly supported as sister to *C. regale* and *C. schiedei*, which have Mesoamerican distributions. The Asian clade is strongly supported as sister to the Hawaiian + Mesoamerican clades.

The sister relationship between the Hawaiian and Mesoamerican clades is supported by spore morphology as described by Gastony (1982). His studies indicated that the spores of Asian species have the greatest degree of exine ridge development, while those of the Hawaiian and Mesoamerican species show an intergradation, with the Mesoamerican species having the least developed ridges.

Based on the phylogeny obtained and the well-supported relationship between the Hawaiian and Mesoamerican clades, there are three possible biogeographic histories. First, it is possible that the ancestral species dispersed to the Hawaiian Islands via the trade winds or storms from Mesoamerica as described by Geiger *et al.* (2007). Second, it is possible that the Hawaiian Islands were colonized before Mesoamerica by an Asian species reaching the Hawaiian Islands via the jetstream. Subsequently, there could have been dispersal of the established Hawaiian species to Mesoamerica. Third, there could have been two independent dispersal events of the same ancestral species out of Asia, one to the Hawaiian Islands and the other to Mesoamerica. This scenario seems much less likely than either of the other two.

Geiger *et al.* (2007) reviewed molecular phylogenies and biogeographical histories of six genera that are represented in the Hawaiian Islands by endemic species. For each of the endemic lineages, they analyzed molecular phylogenies to develop hypotheses about the potential climate/weather-based pathway utilized to disperse to the Hawaiian Islands. They hypothesized that the northern subtropical jetstream was the pathway responsible for delivering ancestral spores of three of five endemic lineages of *Dryopteris* (Geiger and Ranker, 2005), the two endemic *Polystichum* lineages (Driscoll and Barrington, 2007), potentially one lineage of *Hymenophyllum* (Ebihara *et al.*, 2004; Hennequin *et al.*, 2006), and maybe one lineage of *Grammitis* (Ranker *et al.*, 2004; Geiger *et al.*, 2007) to the Hawaiian Islands. The trade winds or storms were the implicated pathways responsible for delivery of *Adenophorus* (Ranker *et al.*, 2003) and possibly of *Stenogrammitis* to the Hawaiian Islands from the Neotropics. The Hadley Cell shift mechanism (Wright *et al.*, 2001) could not be eliminated as a possibility for either *Grammitis* or *Stenogrammitis*.

As stated above, we are unable to eliminate either Asia or Mesoamerica as the source area of the *Cibotium* spores that colonized the Hawaiian Islands based on the topology presented here. Our results suggest it is possible that spores were delivered from Asia via the northern subtropical jetstream or from Mesoamerica via storms or the trade winds to the Hawaiian Islands. Even though the large amount of molecular data used has allowed us to produce a well-supported topology, the relationships resolved in this study do not allow us to favor one dispersal hypothesis over the other. However, a recent biogeographical study of scaly tree ferns, Cyatheaceae, by Korall and Pryer (unpublished data) estimated clade divergence times, and included one Asian, one Mesoamerican, and one Hawaiian *Cibotium* species in the analyses. The dating results suggest that the crown group of *Cibotium* originated 24–25 Ma ago, and the Mesoamerican and Hawaiian clades separated from each other about 17–18 Ma ago. Since Price and Clague (2002) estimated that appropriate Hawaiian montane habitats would not have been present prior to about 5.2 Ma ago when Kauai formed, the hypothesized dispersal route of Asia to the Hawaiian Islands to Mesoamerica via the jetstream to explain the sister relationship of the Hawaiian and Mesoamerican clades would seem unlikely and a biogeographical explanation of dispersal from Asia to Mesoamerica to the Hawaiian Islands via the trade winds or a storm should be favored.

TABLE 3. Geographic distribution and elevation ranges for extant *Cibotium* species.

Species	Native distribution	Elevation range	Reference
Cibotium arachnoideum	SE Asia	Lower montane forest in East Malaysia, 700–2100 m on Mt. Kinabalu [Borneo]	Large and Braggins, 2004
Cibotium barometz	SE Asia	200–1700 m	Large and Braggins, 2004
Cibotium chamissoi	Hawai'i – all major islands except Kaua'i	150–1200 m, as low as 50 m, less common above 800 m	Palmer, 2003
Cibotium cumingii	SE Asia	no information	no information
Cibotium glaucum	Hawai'i – all major islands	300–1700 m, as low as 100 m; is the most common *Cibotium* above 800 m	Palmer, 2003
Cibotium menziesii	Hawai'i – all major islands	250–1400 m	Palmer, 2003
Cibotium nealiae	Hawai'i – Kaua'i only	135–1300 m	Palmer, 2003
Cibotium regale	Mesoamerica	700–2900 m	Davidse *et al.*, 1995
		1140–2200 m	Mickel and Smith, 2004
Cibotium schiedei	Mesoamerica	600–1400 m	Mickel and Smith, 2004

Although the above argument would support a Mesoamerican origin for Hawaiian *Cibotium*, we do not feel we can favor that hypothesis over an Asian origin hypothesis for two reasons. First, although extant species of *Cibotium* seem to favor somewhat higher elevations (500–2500 m), for example in submontane forests (Large and Braggins, 2004), all of the Hawaiian species are reported to occur at 250 m or lower, and *C. chamissoi* is known to occur as low as 50 m (Table 3), thus early colonists (at least based on elevation) theoretically could have survived on the lower elevation islands that existed before the formation of Kaua'i. Much like what was shown for "diellia", a Hawaiian endemic clade of *Asplenium* (Schneider *et al.*, 2005), *Cibotium* could have been one of the early colonizers of Kaua'i having dispersed from an adjacent, lower elevation island. Based on the estimated 17–18 Ma ago split between Mesoamerican and Hawaiian clades, it is possible an ancestral Hawaiian species could have also dispersed to Mesoamerica during that time period. In support of this hypothesis, Hutsemékers *et al.* (2011) have shown that at least some spore-producing plants do not lose their ability to disperse as seed plants often do when they become established on oceanic islands. Second, Vernon and Ranker (2013) have estimated that for the fern and lycophyte taxa that have been studied, the largest proportion of Hawaiian taxa have their closest relatives in the old world, presumably due to the greater efficiency of the jetstream in delivering spores to the Hawaiian Islands compared to the possible mechanisms from other regions. Sixty-one percent of native Hawaiian

taxa were estimated to have their closest relatives in Asia/Paleotropics, whereas only 13% have their closest relatives in the Americas/Neotropics.

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Gametophyte Morphology and Development of Three Species of *Cyrtogonellum* Ching (Dryopteridaceae)

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ABSTRACT.—Gametophyte morphology and development of three species of *Cyrtogonellum* Ching (Dryopteridaceae) were described with the aim to increase our knowledge on the gametophytic stage of this fern genus especially the occurrence of sexual structures. Spore germination followed the *Vittaria*-type development, whereas prothallus growth followed the *Aspidium*-type development. Unicellular glandular hairs were located on both margins and surface of the prothalli. The heart-shaped prothalli of *C. caducum* showed irregularly lobed margins with multicellular filamentous to wing-like extensions. One to several agamously formed embryos were found on a single mature gametophyte of *C. inaequale* but only one of these developed into a mature sporophyte. Young sporophytes possessed scales with one to several glandular cells. Antheridia were found in all three species, but archegonia were observed only on mature prothalli of *C. fraxinellum,* which were always located near the midrib of the cordate-thalloid prothallus. The rarity of archegonia and lack of evidence for the functionality of archegonia in these species raised questions about sexual reproduction in *Cyrtogonellum.*

KEY WORDS.—apogamy, apomixis, *Cyrtogonellum*, polystichoid ferns, reproductive biology

Characters of the gametophytes, including types of spore germination, developmental characteristics, mature gametophyte form, presence and/or absence of trichomes, and ontogeny of the gametangia have been considered to be significant for understanding both the phylogeny and the population biology of pteridophytes (e.g., Atkinson and Stokey, 1964; Nayar and Kaur, 1971; Atkinson, 1973; Farrar *et al.,* 2008). Comparative morphology of the fern gametophyte has provided evidence to elucidate fern relationships by considering gametophyte characters on their own or in combination with sporophyte characters (e.g., Bower, 1923, 1926, 1928; Stokey, 1951; Schneider *et al.,* 2009). In addition, observations of gametophytes have been found crucial in determining modes of reproduction especially the occurrence of apomixis (= apogamous reproduction) in ferns (e.g., Sheffield, 2008; Huang *et al.,* 2011). In this study, we investigate the gametophyte morphology and development of the poorly known fern genus *Cyrtogonellum* Ching (Dryopteridaceae) to better understand its reproductive biology.

The genus *Cyrtogonellum* (Ching, 1938) includes up to nine species and one putative hybrid (Fig. 1A–F; Liu and Zeng, 2010; Liu *et al.*, 2010). All species of the genus are endemic to the karst regions of southern China and northern

FIG. 1. Sporophytes of *Cyrtogonellum* Ching in their natural habitat. A. *Cyrtogonellum caducum* Ching in Yunnan Province, China. B. *Cyrtogonellum caducum* Ching in northern Vietnam. C. *Cyrtogonellum caducum* Ching in Hunan Province, China. D. *Cyrtogonellum caducum* Ching in Hunan Province, showing the sori and the absence of a terminal pinna. E. *Cyrtogonellum fraxinellum* (Christ) Ching in Guizhou Province, China. F. *Cyrtogonellum inaequale* (Christ) Ching in Guizhou Province, China.

Vietnam (Liu *et al.*, 2010). The genus was found to be nested within the polystichoid fern clade as putative sister to *Polystichum* sect. *Sphaenopolystichum* (Liu *et al.*, 2010). The phylogenetic results were consistent with established arguments concerning the close relationships to *Polystichum* (Copeland, 1947; Ching, 1978; Tryon and Tryon, 1982; Kramer *et al.*, 1990; Xie, 1990; Tryon and Lugardon, 1991). Limited understanding of the phylogenetic relationships of polystichoid ferns especially *Cyrtogonellum* motivated several studies targeting the discovery of additional morphological, cytological, and

molecular evidence (Lu *et al.*, 2006, 2007; Li *et al.*, 2008; Liu and Zeng, 2010; Liu *et al.*, 2010). This evidence is required to reveal the phylogeny and improve the taxonomy of these ferns (Zhang and He, 2010).

Cytological studies reported three species of *Cyrtogonellum* as triploids with $2n = 3x = 123$ (Lu *et al.*, 2006). This evidence suggests apogamous reproduction as the main reproductive mode of this genus (Lu *et al.*, 2006; Liu *et al.*, 2012). However, other evidence may indicate the occurrence of sexually reproducing *Cyrtogonellum* species. For example, 41 pairs of chromosomes during prophase 1 of meiosis were reported for a specimen identified as *C. fraxinellum* (Tsai and Shieh, 1985), and also some individuals were recognized as a hybrid *Cyrtogonellum* x *rupicula* (Wang and Wang, 2001). Therefore sexual reproduction might occur in the genus as the formation of hybrids required at least one sexually reproducing parent.

Until now, only one study has been conducted to describe the gametophyte stage of *Cyrtogonellum* and only one species, *C. inaequale*, has been studied (Bao *et al.*, 1999). This study reported spore germination of the *Vittaria*-type and prothallus development of the *Aspidium*-type as expected for ferns belonging to the family Dryopteridaceae. The study also provided evidence supporting apomictic reproduction in this species by noting the presence of antheridia but the absence of archegonia.

The present study was undertaken in order to compare the morphology and ontogeny of different species of *Cyrtogonellum* with a focus on their mode of reproduction. We examined three species: *C. caducum* (Fig. 1A–D), *C. fraxinellum* (Fig. 1E), and *C. inaequale* (Fig. 1F). The main objectives were: (1) confirming the uniformity of spore germination and prothallus development among closely related species; (2) documenting the variation of the morphology of mature gametophytes; (3) determining the presence/absence of sexual organs, in particular archegonia. The later evidence together with observation of the development of young sporophytes is considered critical in identifying the mode of reproduction, sexual or apomictic, for each species of *Cyrtogonellum*.

Materials and Methods

Our study involved three individuals of *Cyrtogonellum caducum* Ching, three individuals of *C. fraxinellum* (Christ) Ching, and one individual of *C. inaequale* (Christ) Ching. The seven individuals sampled were collected from different localities. This sampling strategy allowed comparison of the morphological variation among species and between populations of the same species. Locality and voucher information is presented in Table 1. Voucher specimens were deposited in the Herbarium of Qiannan Normal College for Nationalities, Duyun, Guizhou, China. Spores were collected from fertile pinnae and placed in clean paper bags under dry conditions until spores were shed (usually about one week). Spores were separated from fragments of leaves and sporangia stored at about four degree Celsius.

TABLE 1. Collection data

Cyrtogonellum species	Collector/ voucher	Locality	Collection/ germination	Spore number
C. caducum Ching	Guo ZY/GZY-01	Maolan, Libo, Guizhou	2008-11-29/2009-3-30	32
	Guo ZY/GZY-02	Shamuhe, Shibing, Guizhou	2009-5-7/2009-5-18	32
C. fraxinellum (Christ) Ching	Guo ZY/GZY-03	Sifangtan, Duyun, Guizhou	2009-2-25/2009-4-5	32
	Guo ZY/GZY-04	Shenxiandong, Dushan, Guizhou	2008-11-30/2009-4-17	32
	Guo ZY/GZY-05	Shamuhe, Shibing, Guizhou	2009-5-7/2009-5-20	32
C. inaequale (Christ) Ching	Guo ZY/GZY-06	Shamuhe, Shibing, Guizhou	2009-5-4/2009-5-19	32

Spores were cultured in plastic containers filled with soil collected with the sporophytes. This substrate was sterilized at 120–130 degrees Celsius for 30 minutes. The containers were covered with transparent plastic film, in which five to eight small holes were made in order to reduce the risk of contamination and desiccation. The containers were kept in the laboratory and exposed to natural light conditions throughout the experiments. Cultures were moistened with tap water to prevent desiccation. They were periodically examined to record the onset of spore germination, the growth of gametophytes, the formation of antheridia and archegonia, and the onset of the development of sporophytes. The gametophytes were cultured on native soil rather than agar to enable cultivation in conditions more similar to their natural habitats. Cultivation on agar may increase the probability that the obtained observations were biased by the laboratory conditions. For example, it is well known that apogamous reproduction can be induced in cultures of gametophytes grown on artificial media (Cordle *et al.*, 2012).

Samples were examined at one week intervals during the first month, and subsequently every two weeks. Spore morphology and gametophyte morphology was observed and images were captured using a compound microscope (Motic AE32) with a magnification up to 400x. Description of gametophyte structure and development followed terminology established in previous studies such as *Vittaria*-type spore germination and *Aspidium*-type prothallus development (Nayar and Kaur, 1971).

RESULTS

Spore germination.—Spores of the three *Cyrtogonellum* species were either reniform or globose in shape (Fig. 2A–B). They possessed a monolete aperture and cristate ornamentation. There are 32 spores per sporangium and no evidence of aborted spores.

The spores, collected and stored from one week to five months, germinated about five days after sowing. All three species shared the *Vittaria*-type of spore germination (Fig. 2C). The short and hyaline rhizoid appeared first and initially had a wall perpendicular to the polar axis. It was followed by the first

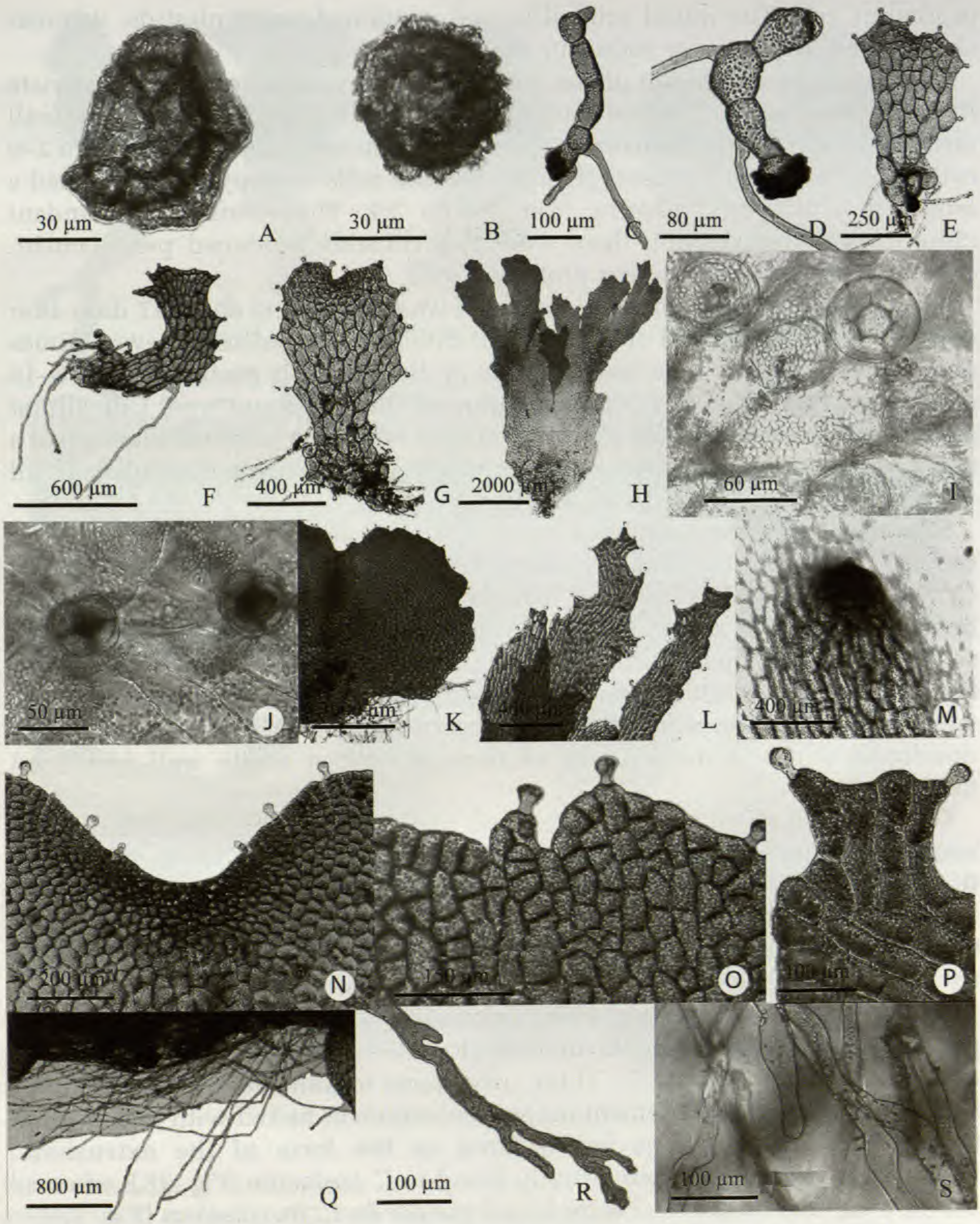

FIG. 2. Spores, prothallia and sexual organs of *Cyrtogonellum fraxinellum* (Christ) Ching. A. Monolete spore. B. Globose spore. C–D. Germinating spore and filamentous phase. E–G. Early stage of prothallus. F. Irregularly lobed mature prothallium. H. Mature, functional antheridia. I. Archegonia. J. Mature, regularly shaped prothallium. K. Irregular outgrowth of prothallia. L. Early stage embryo formed by somatic cells. M. Notch of regularly shaped prothallium. N. Margin of prothallium with papillate, glandular hairs. O–P. Outgrowth of the marginate terminated by glandular hairs. Q. Irregularly shaped rhizoids. R–S. Rhizoids.

prothallus cell. The initial prothallus cell contained many plastids, whereas chloroplasts were rare or absent in the rhizoids (Fig. 2D).

Filamentous and laminar phase.—About nine days after sowing, a uniseriate filament was formed. The first prothallus cell was divided by a transverse wall creating an apical cell. Transverse divisions of the apical cell resulted into 2–6 cells long uniseriate filaments (Fig. 2C–D). The cells were pyriform and had a length to width ratio ranging from 1:1 to 3:1. They contained abundant chloroplasts. Occasionally there were two rhizoids produced per filament. Both were attached to the first prothallus cell.

The differentiation of the laminar phase was observed at about 17 days after sowing. The terminal cell of the filament divided longitudinally several times and formed a slightly lopsided spatulate prothallus. This prothallus was 6–16 cells wide. Prothallus development followed the *Aspidium*-type. Unicellular glandular hairs were formed along the margin while the meristem developed a notch and two wings. These young prothalli were elongate-spathulate in all three species (Fig. 2E–H, 3A–C, 4A–B).

Prothallus.—Prothalli emerged ca. 60 days after sowing. Mature prothalli of the three species differed in shape (Fig. 2E, 2K, 3D, 4C). Prothalli of *Cyrtogonellum fraxinellum* had elongate-spatulate shape with irregularly branched wings, a dense cover of rhizoids, and usually one (Fig. 2E) or sometimes several meristems. The prothalli of *C. caducum* were spatulate with fewer rhizoids, a slightly irregular margin, and a single meristem (Fig. 3D), while those of *C. inaequale* were symmetrically cordiform with two fully developed wings, a dense cover of rhizoids, and a single well-developed meristem (Fig. 4C).

Gametophyte of all three species had papillate-glandular hairs along the margins and on their lower surface (Fig. 2E–G, 2L, 2N–P, 3A–C, 3H–J, 4A–B, 4F, 4H–I, 4K). The globose apices of the papilli were 29–30 μm in diameter. Sometimes the tip was forked resulting in two apical papillae (Fig. 4K). Chloroplasts were located mainly at the center and basal region of the trichomes, which degenerated as the trichomes matured.

Uni-seriate to multi-seriate, lobed extensions were found on the margin of prothalli of *Cyrtogonellum fraxinellum* (Fig. 2O–P), *C. caducum* (Fig. 3J–K), and *C. inaequale* (Fig. 4H–I). These extensions terminated with a glandular hair that was unforked, filamentous and uniseriate or forked with two or more basal cells. The three species differed in the form of the extensions. Filamentous outgrowths were usually found in *C. caducum* (Fig. 3K), whereas lobed, wing-like outgrowths were found mainly in *C. fraxinellum* (Fig. 2H).

Rhizoids were found on the prothalli of all three species. The primary rhizoid arose from germination of the spore while secondary rhizoids were produced by cells of the filament and the prothallus. Rhizoid formation was usually restricted to the ventral surface and adjacent regions of the adult prothallus. Occasionally swollen and forked rhizoids were detected in *Cyrtogonellum fraxinellum* (Fig. 2Q–S) and *C. caducum* (3L–M).

Reproductive organs and young sporophytes.—Prothalli of all three species produced antheridia (Fig. 2I, 3E, 4D–E) but no archegonia were observed for

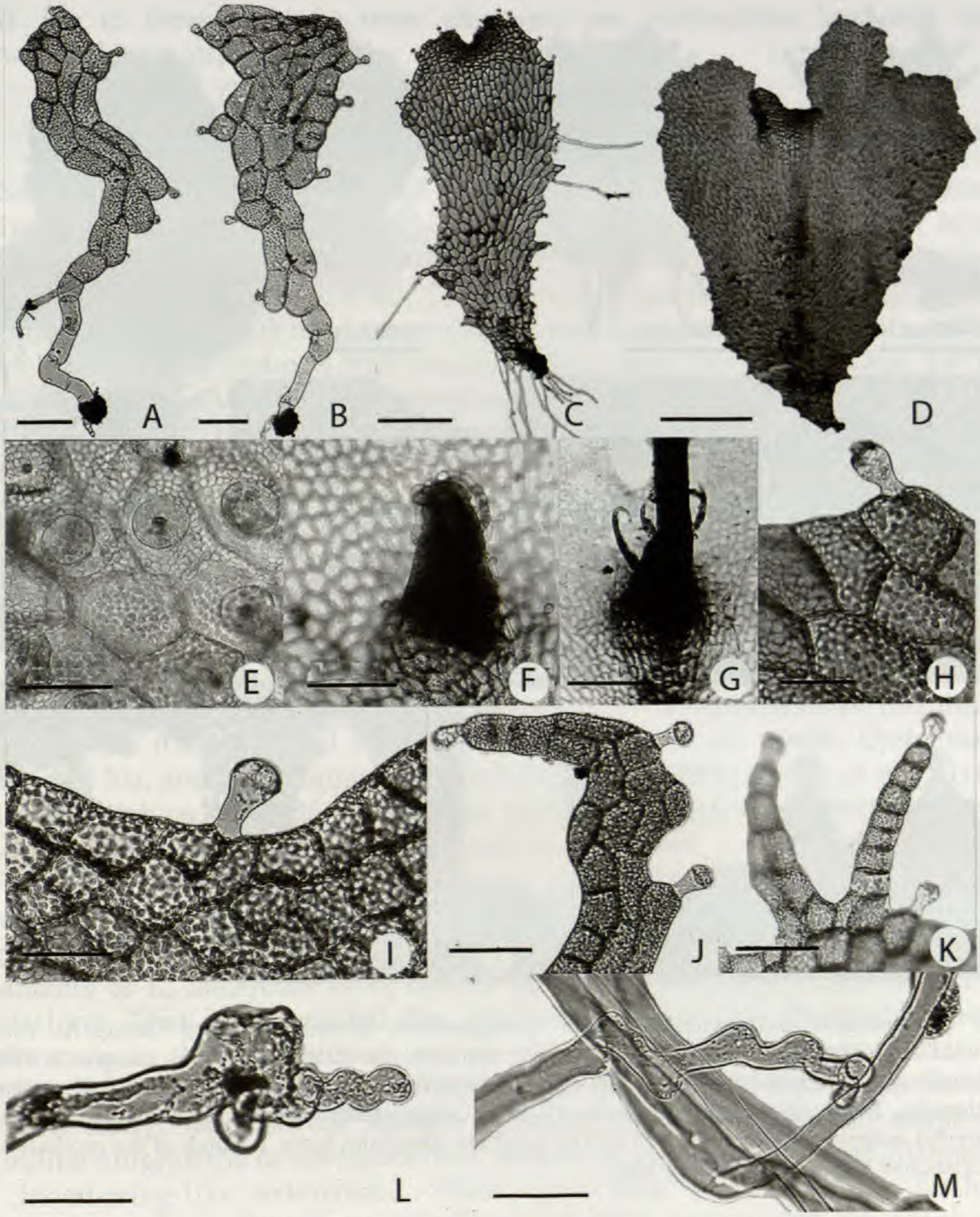

FIG. 3. Prothallia of *Cyrtogonellum caducum* Ching. A–C. Early stage of prothallia. D. Mature prothallium. E. Antheridia. F–G. Early stage embryos originated from somatic cells. H–I. Papillate glandular hairs in the margin. J. Papillate marginal hairs with marginal outgrowth. K. Filamentous marginal outgrowth with terminal, glandular hairs. L–M. Rhizoids.

Cyrtogonellum caducum and *C. inaequale* during the whole period of observations. Prothalli with antheridia were observed from about 60 days after spore sowing. Typically, antheridia appeared on the ventral surface of the prothalli, accompanied by rhizoids or along the midrib of larger prothalli. Mature antheridia were globose and composed of three cells, a basal cell, an

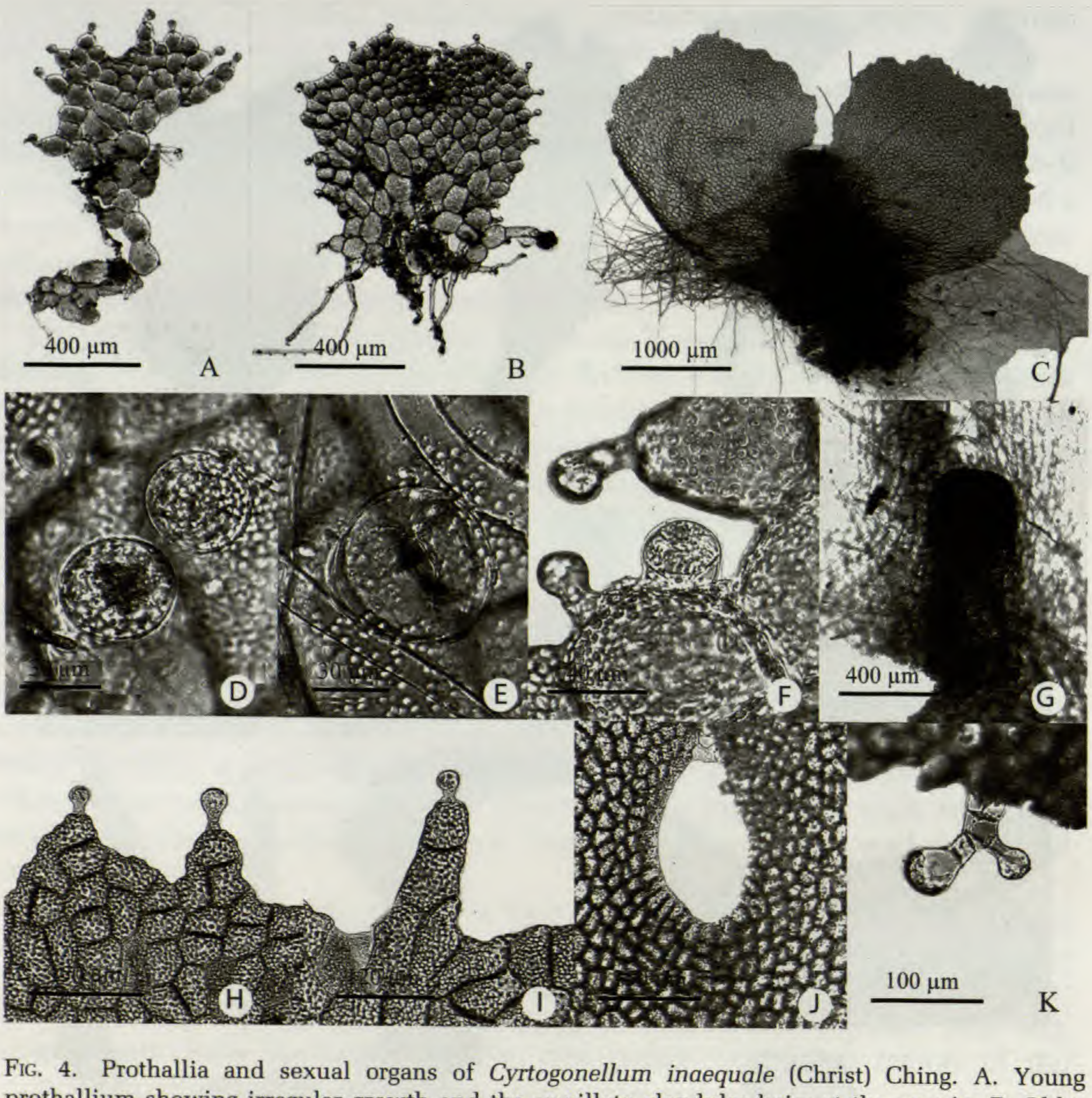

FIG. 4. Prothallia and sexual organs of *Cyrtogonellum inaequale* (Christ) Ching. A. Young prothallium showing irregular growth and the papillate glandular hairs at the margin. B. Older prothallium. C. Mature prothallium showing a regular heart shaped prothallium. D–E. Functional antheridia. F. Papillate glandular hairs. G. Early stage embryo formed by somatic cells. H–I. Marginal outgrowth (filaments) that end in papillate, glandular hairs. J. Notch of the prothallium. K. Branched papillate, glandular hair.

annular or ring cell, and an opercular cell. Spermatozoids were liberated by detachment of the operculum. After release, the spermatozoids were observed actively swimming.

Mature prothalli of *Cyrtogonellum fraxinellum* produced both antheridia and archegonia at the same prothallium (Fig. 2J). Up to a maximum of five archegonia were located near the midrib on the cordate-thalloid prothallium. The terminal neck of the archegonia became brown with age. Mature prothalli of all three species produced sporophytes. These embryos were formed by outgrowth of somatic cells without the involvement of archegonia (Fig. 3F,

4G). Up to three embryos were observed per prothallium but only one developed into a sporophyte.

Discussion

Several observed characters are consistent with the phylogenetic relationships of *Cyrtogonellum* inferred by using DNA sequence data (Liu *et al.*, 2010; Zhang and He, 2010). Spore shape and ornamentation fit the variation reported for the family Dryopteridaceae (Tryon and Lugardon, 1991). The spore germination is of the *Vittaria*-type (Nayar and Kaur, 1971; Bao *et al.*, 1999), which is the most common type among derived ferns (Nayar and Kaur, 1971). The prothalli of *Cyrtogonellum* develop following the *Aspidium*-type (Nayar and Kaur, 1971). This observation is not only consistent with the reports on the gametophyte development of congeneric species, *C. inaequale* (Bao *et al.*, 1999) but also with reports to the vast majority of genera of Dryopteridaceae (Mendoza *et al.*, 1999, 2002; Zhang *et al.*, 2011), including *Cyrtomium* (Chandra and Nayar, 1970; Guo and Yu, 2009), *Phanerophlebia* (Mendoza-Ruiz and Pérez-García, 2003) and *Polystichum* (Chandra and Nayar, 1970; Atkinson, 1973; Pangua *et al.*, 2003; Nazario and Arreguín, 2007; Gabriel y Galán and Prada, 2010; Migliaro and Gabriel y Galán, 2012). Unicellular glandular hairs have been frequently reported for gametophytes of Dryopteridaceae, including *Arachniodes* (Chandra and Nayar, 1970; Mendoza *et al.*, 1999), *Cyrtomium* (Guo and Yu, 2009), *Dryopteris* (Pérez-García *et al.*, 2001; Zhang *et al.*, 2011), and *Polystichum* (Chandra and Nayar, 1970; Nayar and Kaur, 1971; Nazario and Arreguín, 2007; Gabriel y Galán and Prada, 2010).

The early development of the prothalli shows the formation of uniseriate filaments of 2–10 cells although they are less prominent than those reported for *Cyrtogonellum inaequale* (Bao *et al.*, 1999). Bao *et al.* (1999) found filaments of *C. inaequale* to be mostly 30–50 but sometimes more than 100 cells long. They also reported the occurrence of biseriate filaments. These differences in the observations may be either the result of genetic variation within the taxa or caused by differences in the culture conditions. In the current study, we observed notable variation in the number and shape of marginal outgrowths in *Cyrtogonellum* ranging from short uniseriate filaments to lobed-wing-like extensions. Their taxonomic value requires further investigation by cultivating prothalli grown from spores that are obtained from a range of populations and are cultivated under uniform conditions.

The adult prothalli of the three species differ in their growth form. *Cyrtogonellum inaequale* shows regularly formed cordate prothalli with nearly no marginal outgrowth (Fig. 4C). Prothalli of *C. caducum* are slightly asymmetrical cordate with one wing larger than the other (Fig. 3D), and those of *C. fraxinellum* range from regularly cordate (Fig. 2K) to irregularly lobed (Fig. 2H). The irregular form of the prothalli of the last species is likely caused by the extent of marginal outgrowth formed by these prothalli. These observations suggest variation in the developmental pattern among the three studied species. All species have been cultivated under similar conditions,

which rules out effects of differences in cultivation conditions. The prothalli of *C. inaequale* appear to be close to the regular type of prothalli reported in Dryopteridaceae, whereas those of *C. fraxinellum* differ by the occurrence of irregularly shaped wings. The prothalli of *C. caducum* deviate from symmetrical prothalli found in the majority of Dryopteridaceae but similar gametophyte shapes are found in young prothalli and/or male gametophytes in response to antheridogenes (see Schneller, 2008). It is possible that culture conditions may have influenced the observed gametophyte shapes (Nayar and Kaur, 1971; Zhang and Shi, 2005; Deng *et al.*, 2009).

The morphology of the sex organs of the studied *Cyrtogonellum* taxa is clearly of types known to occur in derived leptosporangiates (e.g., Schneider *et al.*, 2009). Only antheridia are formed in *C. caducum* and *C. inaequale*, whereas *C. fraxinellum* possess both antheridia and archegonia. All three species are able to form embryos without the involvement of archegonia. These observations support the notion that some species of *Cyrtogonellum* reproduce via apomixis (= apogamy) and not sexually. Apomixis has been suggested as the common reproductive mode of this genus based on cytological evidence, spore number (Lu *et al.*, 2006) and direct observations of gametophyte growth and development (Bao *et al.*, 1999; Liu *et al.*, 2012). Only a few prothalli of *C. fraxinellum* are observed with archegonia but our observations do not provide evidence for functional archegonia. Future work focusing on the detection of functional archegonia will be conducted because non-functional archegonia have also been reported for apomictic ferns such as *Pteris cretica* (Laird and Sheffield, 1986). In our study, all embryos are found to originate from somatic cells of the prothalli. The loss of functional archegonia in sexually reproducing ferns is considered as the putative trigger for the establishment of obligate apomictic ferns (Moggie, 1990).

All three studied species are reported to be triploids (2n = 3x = 123) with 32 spores per sporangium as shown by Lu *et al.* (2006) and verified by our spore counts. Bao *et al.* (1999) has confirmed apomictic reproduction for *Cyrtogonellum inaequale*, whereas *C. caducum* and *C. fraxinellum* are inferred previously as apomicts based on their chromosome number and the number of spores per sporangium. In general, 32 spores per sporangium have been considered as an indicator for apomictic reproduction in derived ferns. However, it has been argued that observations on the occurrence of sexual organs and the development of young sporophytes are required to confirm the hypothesis of apomictic reproduction (Huang *et al.*, 2011; Liu *et al.*, 2012). In *C. caducum* and *C. inaequale*, the lack of archegonia may be considered as evidence confirming apomictic reproduction. This is consistent with the fact that all observed sporophytes are formed from somatic cells.

Many apomictic ferns possess antheridia but archegonia are usually absent (Manton, 1950; Walker, 1966; Liu *et al.*, 2012). However, non-functional archegonia have been reported for some apomictic fern prothalli (Laird and Sheffield, 1986; Chiou *et al.*, 2006). Thus, the presence of archegonia is insufficient evidence to claim sexual reproduction in *Cyrtogonellum fraxinellum*. However, we need to consider the possibility of karyological variation

in this species. At the current stage, we cannot exclude the occurrence of non-triploid karyotypes in *C. fraxinellum*. Diploid karyotypes may be rare and/or restricted in their distribution in comparison to triploids as shown by studies on other apomictic ferns (e.g., Dyer *et al.*, 2012).

The occurrence of sexual organs and well-formed monolete spores suggest that apomictic *Cyrtogonellum* possess a Döpp-Manton pathway in the production of unreduced spores (Manton, 1950; Walker, 1966, 1985). However, some spores were found to be globose which may be interpreted as an indicator for the Braithwaite pathway (Walker, 1985). Variation of spore shapes has been previously recorded for apomictic fern species (Martinez and Morbelli, 2009) and may reflect instability in the processes of cell divisions leading to the production of unreduced spores.

In conclusion, this study recovered considerable variation among the three species studied. Several key features of the morphology and development are consistent with the phylogenetic relationships of *Cyrtogonellum* and confirm the conservation of developmental processes in the ontogeny of gametophytes in Dryopteridaceae. Due to our limited taxonomic sampling, we cannot draw conclusions on the taxonomic value of prothallus development. The results support the need to investigate these characters in extended studies. More important, our results support the notion that the majority of *Cyrtogonellum* species reproduce via apomixis. We recovered evidence of the formation of functional antheridia, but only one species form archegonia. However, the functionality of these archegonia need further investigation. All three species develop embryos from somatic cells of the gametophyte. So far, evidence for sexual reproduction is lacking for all species belonging to the genus despite the likelihood that sexual reproduction occurred in the common ancestor as indicated by the reproductive biology of related species of polystichoid ferns (Liu *et al.*, 2012).

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Taxonomic Reassessment and Conservation Status of Three Kaua'i Species of *Asplenium* in the *Diellia* Alliance

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Abstract.—Morphological variation of three Kaua'i species of *Asplenium* in the *Diellia* alliance is evaluated based on recent field observations and herbarium specimens. Their taxonomy, nomenclature, and synonymy are discussed, and a lectotype is selected for *Lindsaya knudsenii*. *Asplenium diellaciniatum* is interpreted as having extremely variable frond morphology and dissection, whereas *A. dielmannii* and *A. dielpallidum* are morphologically much more uniform. Their conservation status and population sizes are reviewed.

Key Words.—Aspleniaceae, *lectotypification*, *morphology*, Kaua'i, Hawai'i, conservation, taxonomy

The Hawaiian endemic fern genus *Diellia* Brack. was previously considered to include six species (Palmer 2003; Wagner 1952, 1993). The origin of this group of six species is thought to date to ca. 2 Myr ago and coincide with the renewal of Hawaiian terrestrial life in the Miocene (Schneider et al. 2005a). However, recent molecular phylogenetic studies have shown *Diellia* to be deeply nested within *Asplenium* L. (Schneider et al. 2005b), and all names in *Diellia* have been transferred to or given new names in *Asplenium* (Viane & Reichstein 1991; Snow 2011; Snow et al. 2011). However, the new names or combinations were made by these authors essentially to provide valid names for these taxa in *Asplenium* based on Palmer's and Wagner's species concepts, without any further taxonomic evaluation. Consequently, several of the species complexes in this group have not yet been satisfactorily resolved taxonomically, including one with three species on Kaua'i. Recent intensive field work on Kaua'i by the second and third authors has revealed the presence of new populations of taxa in this rare *Asplenium* alliance. Observations and specimens deposited in the National Tropical Botanical Garden Herbarium (PTBG) have helped reassess the considerable morphological variation in this complex. We here discuss the taxonomy, nomenclature, and synonymy of the three Kaua'i species in this alliance and select a lectotype for *Lindsaya knudsenii*.

As currently circumscribed, three species in this alliance occur on Kaua'i, separable by characters in the key below. They are: *Asplenium dielmannii*

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Viane, *A. dielpallidum* Viane, and *A. diellaciniatum* Viane. The first two species are relatively uniform morphologically and easily recognized. However, population studies of the third species reveal an amazing range of variation in leaf morphology and degree of lobing. Hillebrand (1888) named four different species and six varieties (not all were validly published) in the genus *Lindsaya* Kaulf. [= *Lindsaea* Sm.] in order to encompass this astonishing array of variation: *Lindsaya alexandri* (Hillebr.) Hillebr. including one variety from Kaua'i, var. *bipinnata* Hillebr. (fronds bipinnate); *Lindsaea centifolia* Hillebr. from Kaua'i; *Lindsaea knudsenii* Hillebr. from Kaua'i; and *Lindsaea laciniata* Hillebr. from Kaua'i, with var. subpinnata (fronds subpinnate).

Except for the two varieties of *Lindsaya alexandri* from Maui, all the specimens Hillebrand used to typify these names were collected from essentially the same vicinity on Kaua'i, called "Halemanu" by island resident Valdemar Knudsen who sent plant specimens to Hillebrand. The specimens were subsequently deposited in the Berlin herbarium and images are now available online (Röpert 2000-present, http://www.bgbm.org/). Halemanu was the site of the Knudsen family's mountain home in western Kaua'i. Searches in the vicinity of the old Knudsen homestead have failed to reveal any extant populations of *Asplenium diellaciniatum* around Halemanu (K. Wood, pers. obs.) However, two new populations numbering ca. 90 plants were recently discovered at Kawai'iki, located about 7.25 km from Halemanu in a similar habitat type, *Acacia koa* A. Gray mesic forest.

Study of these two subpopulations at Kawai'iki and additional herbarium collections reveals that these morphotypes are neither consistent nor stable and intergrade with each other. We conclude that a single species, *Asplenium diellaciniatum,* is represented with extremely variable frond morphology and dissection, depending on age and stage of development of the plant and possibly also microhabitat (K. Wood and R. Aguraiuja, pers. obs.). A single plant may even possess fronds representing several morphotypes corresponding to taxa described by Hillebrand (e.g., *Wood & Query 1174, 1175, 1177,* all PTBG; *Wood & Perlman 9015, 9060, 9062, 9062B, 9062C,* all PTBG), (Figures 1, 2). A similar situation exists in *A. daucifolium* Lam., a species of Madagascar and the Mascarene Islands which displays a series intergrading frond forms ranging from 1- to 4-pinnate (Tardieu-Blot 2008).

In his seminal study of the morphology and taxonomy of the genus *Diellia,* Wagner (1952) recognized this variation for Kaua'i plants but ascribed it to *Diellia erecta* Brack., as forma *alexandri* (Hillebr.) W. H. Wagner. He recognized two additional species from this region of Kaua'i, *D. mannii* (D.C. Eaton) W. J. Rob. and *D. laciniata* (Hillebr.) Diels, and later described a third species, *D. pallida* W. H. Wagner (1993). Wagner's species concepts were generally followed by Palmer (2003) in his treatment of Hawaiian ferns. Palmer recognized *Diellia pallida* and *D. mannii* [=*Asplenium dielmannii* Viane] as distinct species, although he synonymized Hillebrand's *Lindsaya knudsenii* β var. under the latter. He also subsumed the names *Diellia centifolia* and

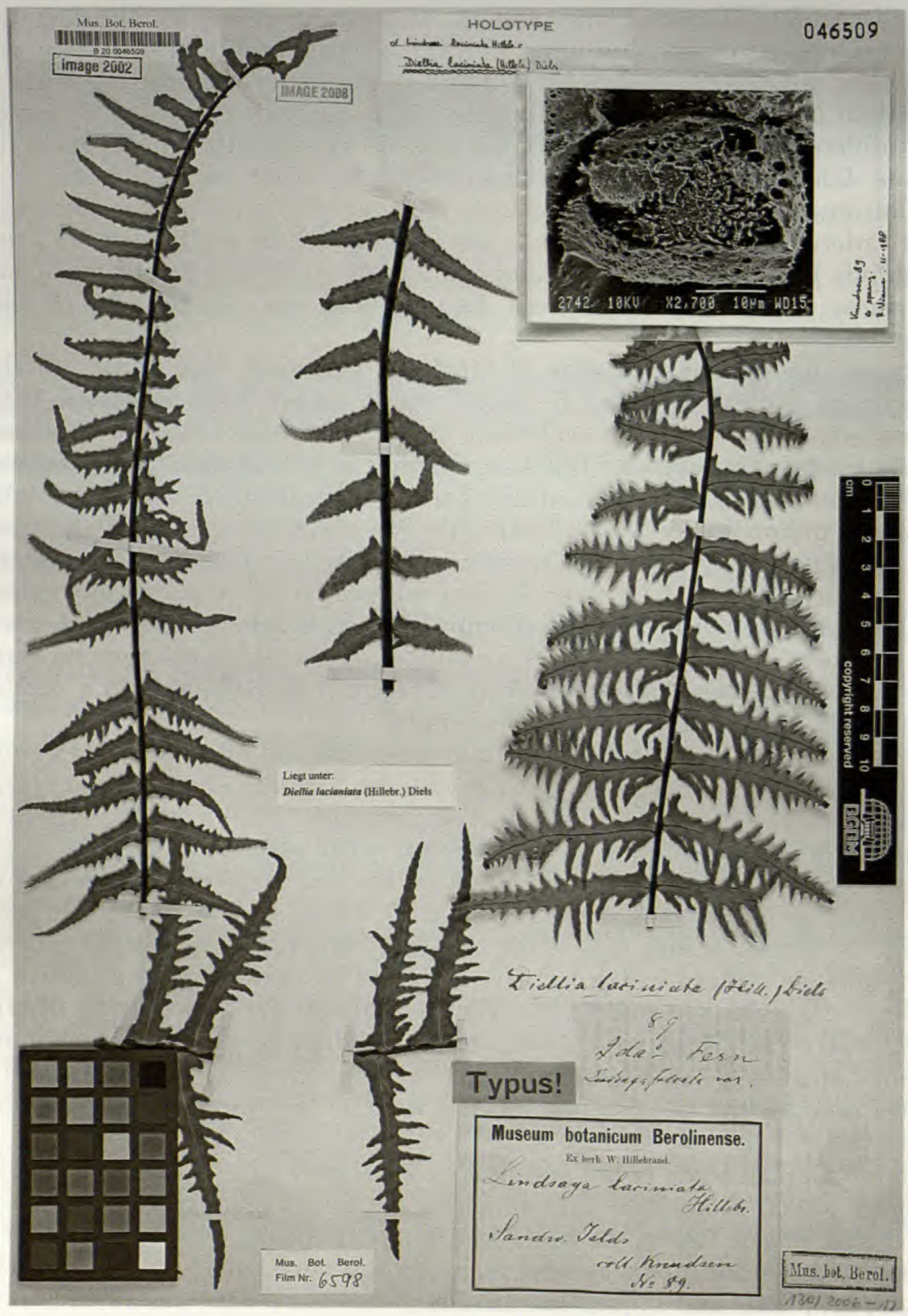

FIG. 1. *Asplenium diellaciniatum*. Holotype of *Lindsaya laciniata* Hillebr. (Knudsen 89, B), courtesy of Röpert, D. (Ed): Digital specimen image at the Herbarium Berolinense. - Published on the Internet http://ww2.bgbm.org/herbarium/ (Barcode: B 20 0046509 / ImageId: 268009) [accessed 29-May-13].

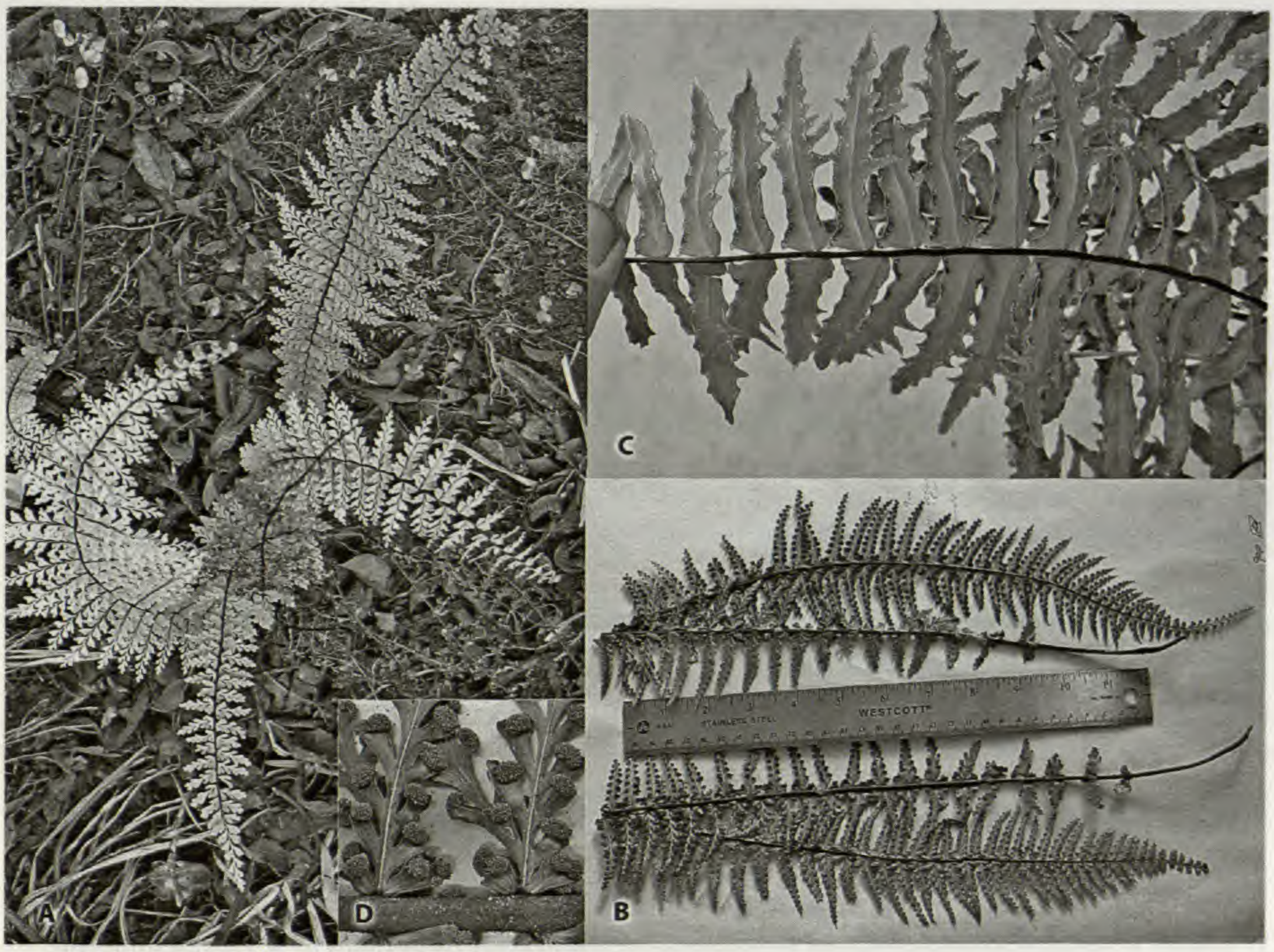

FIG. 2. *Asplenium diellaciniatum.* A, habit, bipinnate-pinnatifid form, Kawai'iki, Kauai'i; B, D, pinnate-pinnatifid form, Kawai'iki, Kaua'i; C, irregularly laciniate pinnate-pinnatifid form, Kawai'iki, Kauai'i. (Photos: A, Ruth Aguraiuja, unvouchered; B, D, *K. R. Wood 9010* (PTBG); C, *D. Lorence 10189,* PTBG, from cultivated plant NTBG # 100358, origin Kawai'iki, Kauai'i.)

Hillebrand's varieties γ and δ of *Lindsaya alexandri* under *Diellia erecta* Brack f. *alexandri* (Hillebr.) W. H. Wagner.

The name *Diellia erecta* Brack f. *alexandri* has been incorrectly applied to Kaua'i plants. Hillebrand's two syntypes of *Diellia alexandri* are from East Maui and Kaua'i and resemble each other superficially, which apparently has given rise to the belief that a single widespread species is involved. In our opinion, however, the two syntypes actually represent two distinct taxa. The Kaua'i syntype (*Knudsen s.n.*, B) is referable to *Asplenium diellaciniatum,* whereas the Maui syntype (*Lydgate & Alexander s.n.*, B) is referable to *A. dielerectum.* Furthermore, the type of *D. erecta* Brack. is from East Maui, suggesting this species is restricted to Maui Nui (Maui, Lana'i, and Moloka'i), an opinion shared by Hillebrand (1888). Wagner (1952: 156) effectively lectotypified *D. erecta* f. *alexandri* [based on *Lindsaya alexandri* Hillebr.] by citing *Lydgate s.n.* from the North flank of Haleakala as "type", thus effectively linking this name to a plant from Maui. Wagner nevertheless believed *D. erecta* f. *alexandri* occurred on Kaua'i as well as Maui, Lana'i, and Moloka'i, noting that the Maui Nui plants have fronds that are also variable in dissection and intergrade, similar to the variability expressed in the Kaua'i plants. The Kaua'i

plants, however, are generally larger with fronds 50–70 cm long and robust stipes 2.5–3.5 mm thick, compared with the Maui Nui plants, which have shorter fronds and thinner stipes 1–2 mm thick. Wagner (1952) separated Kaua'i populations of *D. erecta* from those on the other islands based on their maroon or darker stipes with large gray or tan scales, fronds with 20–40 pinna pairs, the blade apex narrowing abruptly, and the cuneate segments narrow at the base.

Since Wagner linked the epithet *alexandri* to the Maui plants, which we consider to represent a distinct species (*Asplenium dielerectum*), we conclude the name *A. diellaciniatum* Viane [syn. *Diellia laciniata* (Hillebr.) Diels] should be applied to the Kaua'i plants for two reasons. First, the holotype (Fig. 1) is from Kaua'i and corresponds closely to morphotypes in the Kawai'iki population displaying laciniate-pinnatifid lobing of the pinnae. Secondly, although *Lindsaya knudsenii* and *L. laciniata* were published concurrently by Hillebrand (1888), the name *Asplenium diellaciniatum* Viane [based on *Lindsaya laciniata* Hillebr.] is available in *Asplenium* and must be adopted under the International Code of Botanical Nomenclature (McNeill et al., 2012). The three species in the *Diellia* alliance occurring on Kaua'i may be separated by the following key. Nomenclature and synonymy are given below for each species.

Key to Kaua'i *Asplenium* in the *Diellia* Alliance (Adapted from Palmer 2003)

1. Blades 3-pinnate to 3-pinnatifid or 4-pinnate; ultimate pinnule segments narrow, linear or linear-lanceolate, 0.4–1.4 mm wide, thinly chartaceous. . . . . *A. dielmannii*
1. Blades 1- to 2-pinnate (sometimes 3-pinnatifid or completely 3–pinnate in *A. diellaciniatum*); pinnae entire to serrate or laciniate, or ultimate segments usually cuneate if present, 1.5–3(5) mm wide, texture stiffly chartaceous or subcoriaceous. 2
   2. Stipes and rachis usually light to dark brown or pale tan, dull; blades 1-pinnate; pinnae entire; veins usually anastomosing to form 2–3 layers of areoles close to costae near bases of pinnae. . . . . *A. dielpallidum*
   2. Stipes and rachis black or purple-black to maroon, shiny; blades 1–2(3)-pinnate, pinnae entire to shallowly or deeply pinnatifid or pinnate; veins mostly free, seldom anastomosing near costae to form one layer of areoles. . . . . *A. diellacinatum*

1. ***Asplenium diellaciniatum*** Viane, Biol. Jaarb. 59: 159. 1991, nom. nov. for *Lindsaya laciniata* Hillebr., Fl. Hawaiian Isl. 621. 1888, not *Asplenium laciniantum* D. Don. (1825). Type: USA, Hawaiian Islands, Kaua'i, s. loc., "Ida's Fern, Lindsaya falcata var.", *Knudsen 89* (Holotype B-20 0046509, image seen; fragment NY [pinna], image seen).

Lindsaya knudsenii Hillebr., Fl. Hawaiian Isl. 623. 1888. Type: USA, Hawaiian Islands, Kaua'i, Halemanu, Knudsen [as "Kn"] 22 (**lectotype, here designated**: B-20 0046502, image seen; Isolectotype: Knudsen 22, B-20 0046505).

Lindsaya centifolia Hillebr. Fl. Hawaiian Isl. 621. 1888. Type: USA, Hawaiian Islands, Kaua'i, Halemanu, without precise date, Knudsen s.n. [as "Kn"] (Not located).

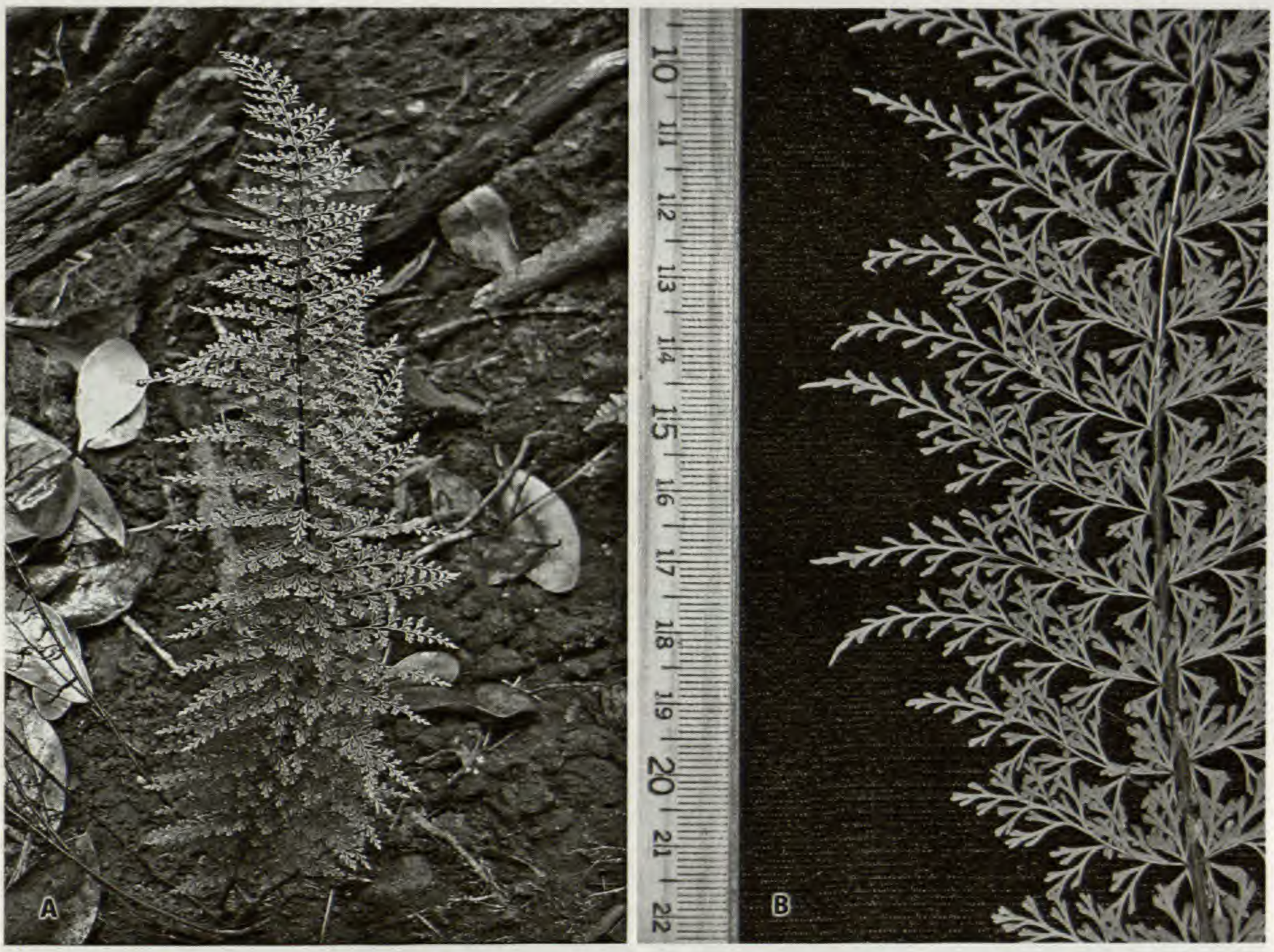

FIG. 3. *Asplenium dielmannii*. A, habit, plant with single frond at Halemanu, Kaua'i; B, detail of frond, Kawai'iki Ridge, Kaua'i. (Photos: A, Ruth Aguraiuja, unvouchered; B, *K. R. Wood 13858*, PTBG.)

Lindsaya alexandri Hillebr. var. *bipinnata* Hillebr., Fl. Hawaiian Isl.: 623 1888. Type: Kaua'i, Halemanu, without precise date, Knudsen s.n. [as "Kn"] (Holotype B, image seen). **Figs. 1, 2.**

Discussion.—The specimen *Knudsen 22* is selected as the lectotype of *Lindsaya knudsenii* because there are two sheets at B consisting of mature, fertile fronds representative of this taxon.

Population status.—Two populations consisting of about 90 reproductive individuals are known in the upper drainage of Kawai'iki Valley, Kaua'i (Aguraiuja, unpublished). Not yet federally listed because of prior taxonomic confusion regarding the nomenclature of this species. However, it is included on the Hawai'i State Plant Extinction Prevention Program (PEP) list http://pepphi.org

2. ***Asplenium dielmannii*** Viane, Biol. Jaarb. 59: 160. 1991, nom. nov. for *Microlepia mannii* D. C. Eaton, Proc. Am. Acad. 7: 212. 1868, not *Asplenium mannii* Hook. (1861). *Diellia mannii* (D. C. Eaton) W. J. Rob., Bull. Torrey Bot. Club 39: 582. 1912. Type: USA, Hawaiian Islands, Kaua'i, Waimea, 2000–3000 ft., *Mann & Brigham 546* (GH, image seen; Isotype US!). **Fig. 3.**

FIG. 4. *Asplenium dielpallidum*. A, habit, plant at Mahanaloa Valley, Kaua'i; B, frond showing mature sori. (Photos: A, Ruth Aguraiuja, unvouchered; B, David Lorence, from cultivated plant NTBG # 070681, origin Mahanaloa Valley, Kaua'i.)

Population status.—This species was formerly considered extinct (Palmer 2003), but was rediscovered on Kaua'i in 2003 (Aguraiuja and Wood, 2003) and is federally listed as endangered. One population consisting of 62 reproductive individuals is known (Aguraiuja, unpublished).

3. ***Asplenium dielpallidum*** N. Snow, Bishop Museum Occasional Papers 110: 12. 2011, nom. nov. for *Diellia pallida* W. H. Wagner, Contr. Univ. Michigan Herb. 19: 66. 1993, not *Asplenium pallidum* Blume (1828). Type: USA. Hawaiian Islands: Kaua'i, Mahanaloa Valley, 10 Aug. 1949, *W. H. Wagner, Jr. 5805* (Holotype MICH 1259996, image seen). **Fig. 4**.

Population status.—Listed as Endangered by the USFWS, the known natural population of this species consists of seven mature individuals in three localities on western Kaua'i (Aguraiuja, unpublished).

Other Hawaiian Species

Other Hawaiian species in the *Diellia* alliance are *Asplenium dielfalcatum* Viane and *A. unisorum* (W. H. Wagner) Viane, both known from O'ahu where

they may hybridize, *A. leucostegioides* Baker from East Maui (presumed extinct), and *Asplenium dielerectum* Viane (all the major islands except Kaua'i, Kaho'olawe and Ni'ihau). Further study is needed to clarify the status of the forms of the Maui Nui plants within *Asplenium dielerectum,* including *Diellia erecta* f. *alexandri* for which the nomenclature and typification are summarized below.

Diellia erecta Brack. f. *alexandri* (Hillebr.) W. H. Wagner, Univ. Calif. Publ. Bot. 26: 155. 1952. Basionym: *Diellia alexandri* (Hillebr.) Diels, Nat. Pflanzenfam. [Engler & Prantl] 1[4]: 212 f. 114 G-K. 1899. *Lindsaya alexandri* Hillebr., Fl. Hawaiian Isl. 622. 1888. Lectotype, designated by Wagner (1952): USA: Hawaiian Islands. Maui: Northern slope of Haleakala, 3000–4000 ft, *Alexander & Lydgate* (Lectotype B, image seen).

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A New Hybrid of *Serpocaulon* (Polypodiaceae) from Costa Rica

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ABSTRACT.—A new hybrid *Serpocaulon* × *sessilipinnum* A. Rojas & J.M. Chaves is described. The new taxon has characters intermediate between *S. fraxinifolium* (Jacq.) A.R. Sm. and *S. ptilorhizon* (Christ) A.R. Sm., which are related mainly with leaf size, rhizome scales, shape and division of blade, shape and width of pinnae, number of areolae and sori series, and shape and color of spores.

KEY WORDS.—Taxonomy, Ferns, Hybridization, Neotropics

Serpocaulon A.R. Sm. is a segregated genus of *Polypodium* L. (Smith et al., 2006), and many of its species were initially described in *Goniophlebium* (Blume) C. Presl, a genus of about 25 species occurring from India, southeast and eastern Asia, the Himalayan region, and Malaysia to Australasia, Melanesia, Samoa, and Micronesia (Röd-Linder, 1990). However, molecular phylogenetic analysis (Schneider et al., 2004) have shown that *Serpocaulon* is nested in a predominantly New World clade, containing the genera *Microgramma* C. Presl, *Campyloneurum* C. Presl, *Pleopeltis* Humb. & Bompl. ex Willd., *Pecluma* M.G. Price and *Polypodium* s.s., as well as the Pantropical grammitid clade (Smith et al., 2006).

Serpocaulon is defined as having commonly anastomosing veins, chevron-shaped areoles, 1–10 series of areoles between the costa and pinnae margin, excurrent veinlets in the areoles, as well as a generally long-creeping, branched rhizome, with peltate, clathrate rhizome scales (at least in the central or basal portion). It also has monolete spores with verrucate and occasionally winged ornamentation (Lloyd, 1969; Coelho and Esteves, 2011; Ramírez-Valencia et al., 2013).

Serpocaulon is entirely Neotropical, containing about 40–45 species (Smith et al., 2006). Of these, 14 are restricted to the West Indies, Central America and southern Mexico; two are confined to the West Indies, North America, Central America and northern Colombia, and 26–28 occur in South America (Smith et al., 2006), with the diversification centers being the Cordillera Central of Colombia (Sanín, 2006) and the Brazilian Atlantic Forest (Schwartsburd and Smith, 2013).

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Smith et al. (2006) divided the genus into four informal groups: 1) *S. loriceum*, 2) *S. fraxinifolium*, 3) *S. subandinum* and 4) *S. lasiopus*. The new hybrid described here, between *S. fraxinifolium* (group 2) and *S. ptilorhizon* (group 3), provides evidence that the limits between these groups are weak.

Serpocaulon* × *sessilipinnum A. Rojas & J.M. Chaves, ***hyb. nov.*** TYPE.—Costa Rica. Puntarenas: Coto Brus, San Vito, Cerro Paraguas, orillas de la laguna Los Gamboa, 8°47′16.3″N, 82°59′20″W, 1447 m, 3 Nov 2012, *J.M. Chaves et al. 299* (Holotype: CR; Isotypes: HLDG, MO) (Figs. 1B and E, 2B and E).

Diagnosis: The new hybrid differs from *S. fraxinifolium* (Jacq.) A.R. Sm. by having rhizome scales that are relatively smaller, moderately dense, with dark brown central portions and narrower pale margins; blades that are 1-pinnate basally to pinnatisect distally and relative narrower; pinnae that are sessile and deltate to deltate-lanceolate; fewer series of areoles and sori, spores that are whitish, ellipsoidal, most well developed and a few collapsed. Similar characters differentiate the new hybrid form *S. ptilorhizon* (Christ) A.R. Sm. Additionally, *S. ptilorhizon* has rhizome scales that are smaller and less dense than the hybrid, and scales have a blackish central portion and a narrower pale margin; blades that are relatively narrower and pinnatisect; pinnae that are lanceolate to narrowly deltate; fewer series of areoles and sori; and spores that are yellow and regularly ellipsoidal.

Description.—Rhizome long creeping, 2.5–4 mm in diameter, non-pruinose, moderately scaly; *rhizome scales* 1–2 × 0.9–1.5 mm, orbicular to ovate, clathrate, dark brown centrally, with yellowish to light brown margin 0.1–0.3 mm, appressed, marginally entire to irregularly-lobulate, apically obtuse to rounded; *fronds* 44–53.5 cm long, separated by 0.5–8.5 cm; *stipe* 21.6–25.2 × 0.1–0.2 cm, ribbed, stramineous to light brown, glabrous except for trichomes 0.2–0.3 mm, sparse, brown; *blade* 22.5–28.2 × 13.6–21 cm, narrowly deltate, 1-pinnate to pinnatisect distally, basally truncate, apically subconform; *pinnae* 1.9–10.3 × 0.5–1.7 cm, 8–13 pairs, linear-lanceolate, straight to falcate, basal pinnae slightly deflexed, marginally entire; *apical pinnae* 3.8–11 × 0.8–1.3 cm, similar to lateral pinnae, with 1–2 basal lobules; rachis and costae stramineous to light brown, sparsely scaly, scales similar to rhizome scales; *laminar tissue* glabrous; *veins* reticulate, forming 2–3 series of areoles between costa and margin; *sori* round, located in (1–) 2 lines between costa and margin; *sporangia* glabrous; *spores* 47–51 × 28–38 μm, bilateral, ellipsoidal, convex to slightly concave-convex, exospore prominently verrucate, verrucae 3–6.2 μm, translucent, some with yellow patches, others irregular.

Additional Specimen Examined.—Costa Rica. Guanacaste: Liberia, Parque Nacional Guanacaste, sector Santa María, sendero que va al volcán Santa María, zona de campamento, 10°48′03″N, 85°19′02″W, 1600-1700 m, 3 Sep. 2012, *A. Rojas et al. 10249* (CR, MO, USJ).

Etymology.—The specific epithet refers to the sessile pinnae.

Serpocaulon × *sessilipinnum* has characters that are intermediate between *S. fraxinifolium* (Jacq.) A.R. Sm. and *S. ptilorhizon* (Christ) A.R. Sm. including

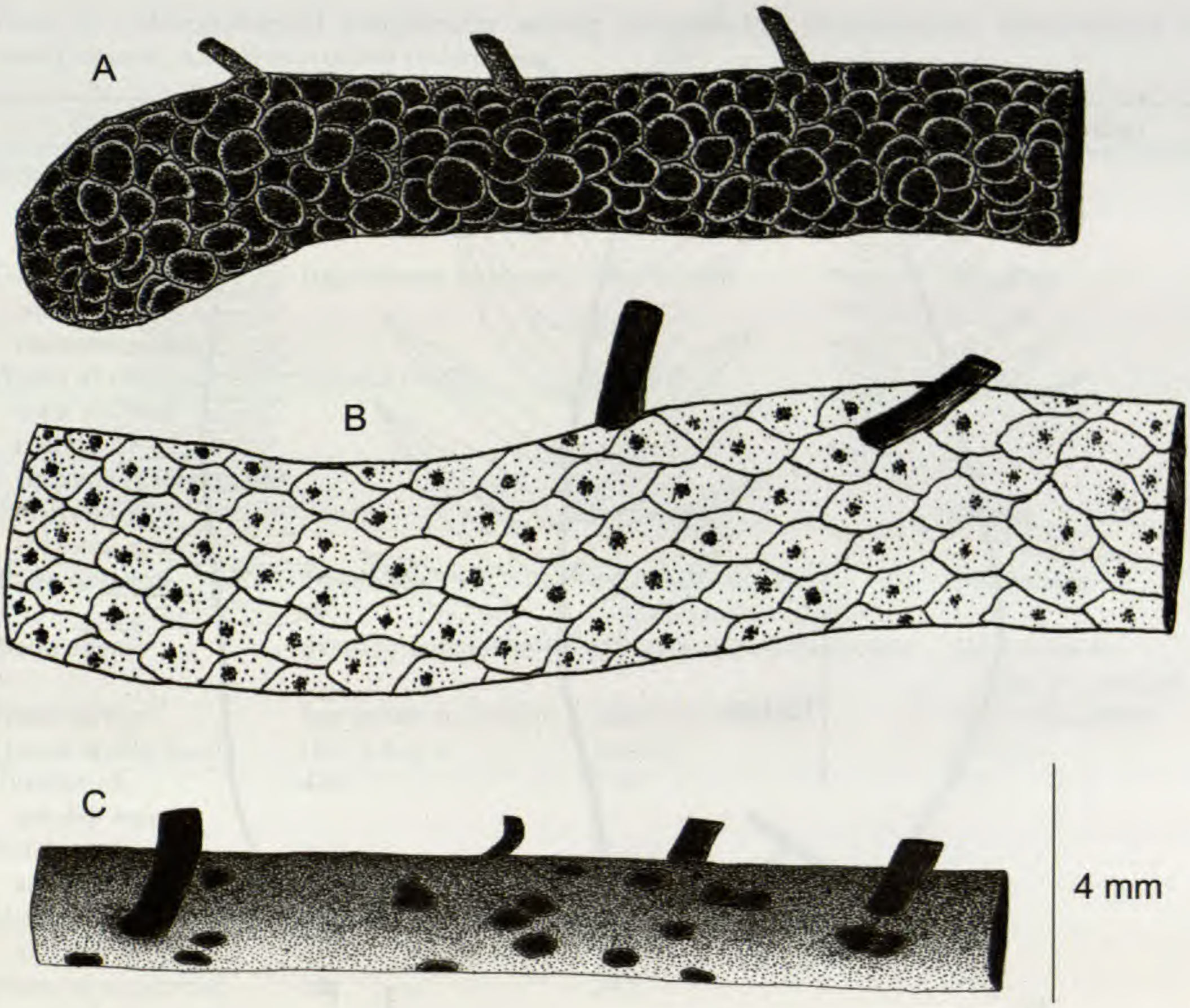

FIG. 1. A and D. *Serpocaulon fraxinifolium* (*J.M. Chaves et al. 297*, CR); A. Habit. D. Blade detail. B and E. *S.* × *sessilipinnum* (*J.M. Chaves et al. 299*, CR); B. Habit. E. Blade detail. C and F. *S. ptilorhizon* (*J.M. Chaves et al. 298*, CR); C. Habit. F. Blade detail.

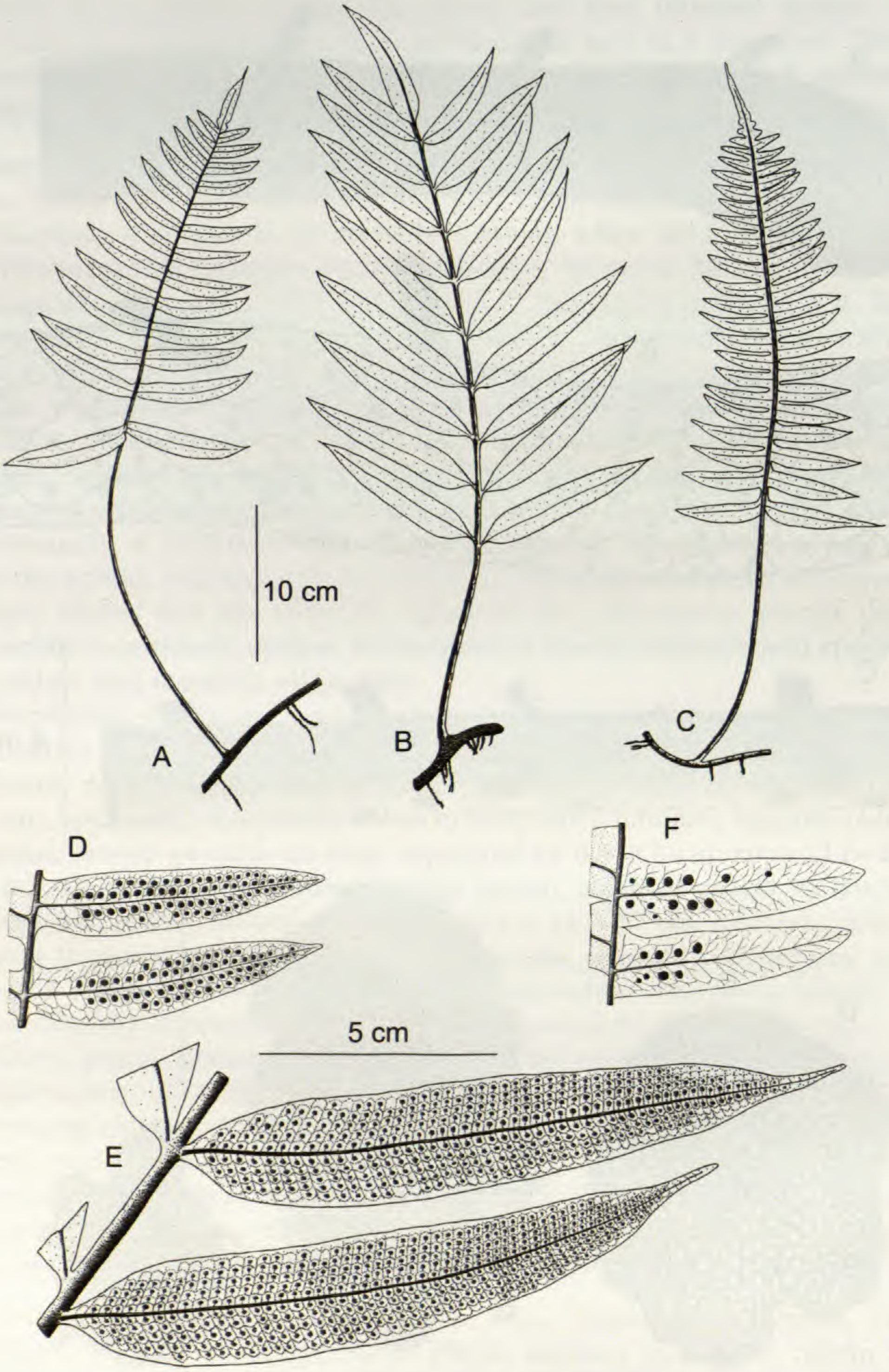

FIG. 2. A and D. *Serpocaulon fraxinifolium* (*J.M. Chaves et al. 297*, CR); A. Rhizome detail. D. Rhizome scale. B and E. *S.* × *sessilipinnum* (*J.M. Chaves et al. 299*, CR); B. Rhizome detail. E. Rhizome scale. C and F. *S. ptilorhizon* (*J.M. Chaves et al. 298*, CR); C. Rhizome detail. F. Rhizome scale.

TABLE 1. Morphological comparison among *Serpocaulon fraxinifolium*, *Serpocaulon* × *sessilipinnum*, and *Serpocaulon ptilorhizon*.

Character	*S. fraxinifolium*	*S.* × *sessilipinnum*	*S. ptilorhizon*
Size of rhizome scales (mm)	(1.5–) 2–3 × (1–) 1.5–2	1–2 × 0.9–1.5	0.5–1
Color of central portion of rhizome scales	light brown to brown	dark brown	Blackish
Width of the light colored margin of rhizome scales (mm)	0.2–0.5 (–0.7)	0.1–0.3	≤ 0.1
Density of rhizome scales	dense	moderate	Sparse
Blade division	1-pinnate	1-pinnate basally to pinnatisect distally	Pinnatisect
Blade shape	ovate to lanceolate	deltate to deltate-lanceolate	lanceolate to narrowly deltate
Pinnae shape	lanceolate to elliptic	linear-lanceolate	linear-lanceolate
Pinnae width (cm)	(1–) 1.8–3.3	0.5–1.7	0.5–1.1
Number of areoles series	4–5	2–3	1–2
Number of sori series	3–5	(1–) 2	1
Mean of polar axis of spore (μm)	30.2	48.8	48.4
Mean of equatorial axis of spore (μm)	50	34.5	72.3
Color of spores	yellow	whitish	yellow
Spore shape	ellipsoidal	ellipsoidal or collapsed	ellipsoidal

size, color of central portion, width of pale margin, density of rhizome scales, shape and division of blade, shape and width of pinnae, number of areolae and sori series, and shape and color of spores (Table 1). The new hybrid differs from *S. fraxinifolium* by having smaller (1–2 × 0.9–1.5 mm vs. (1.5–) 2–3 × (1–) 1.5–2 mm) rhizome scales with dark brown (vs. light brown) central portions and narrower (0.1–0.3 mm vs. 0.2–0.5 (–0.7) mm) pale margins with moderate (vs. high) density, 1-pinnate basally to pinnatisect distally (vs. 1-pinnate throughout) blade divisions, relatively narrower (0.5–1.7 cm vs. (1–) 1.8–3.3 cm), deltate to deltate-lanceolate (vs. ovate to lanceolate) and sessile (vs. free) pinnae, fewer series (2–3 vs. 4–5) of areoles and fewer series ((1–) 2 vs. 3–5) of sori, and whitish (vs. yellow) and ellipsoidal to collapsed (vs. ellipsoidal) spores (Table 1).

From *Serpocaulon ptilorhizon*, *S.* × *sessilipinnum* differs by its longer (1–2 × 0.9–1.5 mm vs. 0.5–1 mm in diameter) rhizome scales with dark brown (vs. blackish) central portion and broader (0.1–0.3 mm vs. ≤0.1 mm) pale margin, moderate (vs. sparse) scale density, 1-pinnate basally to pinnatisect distally

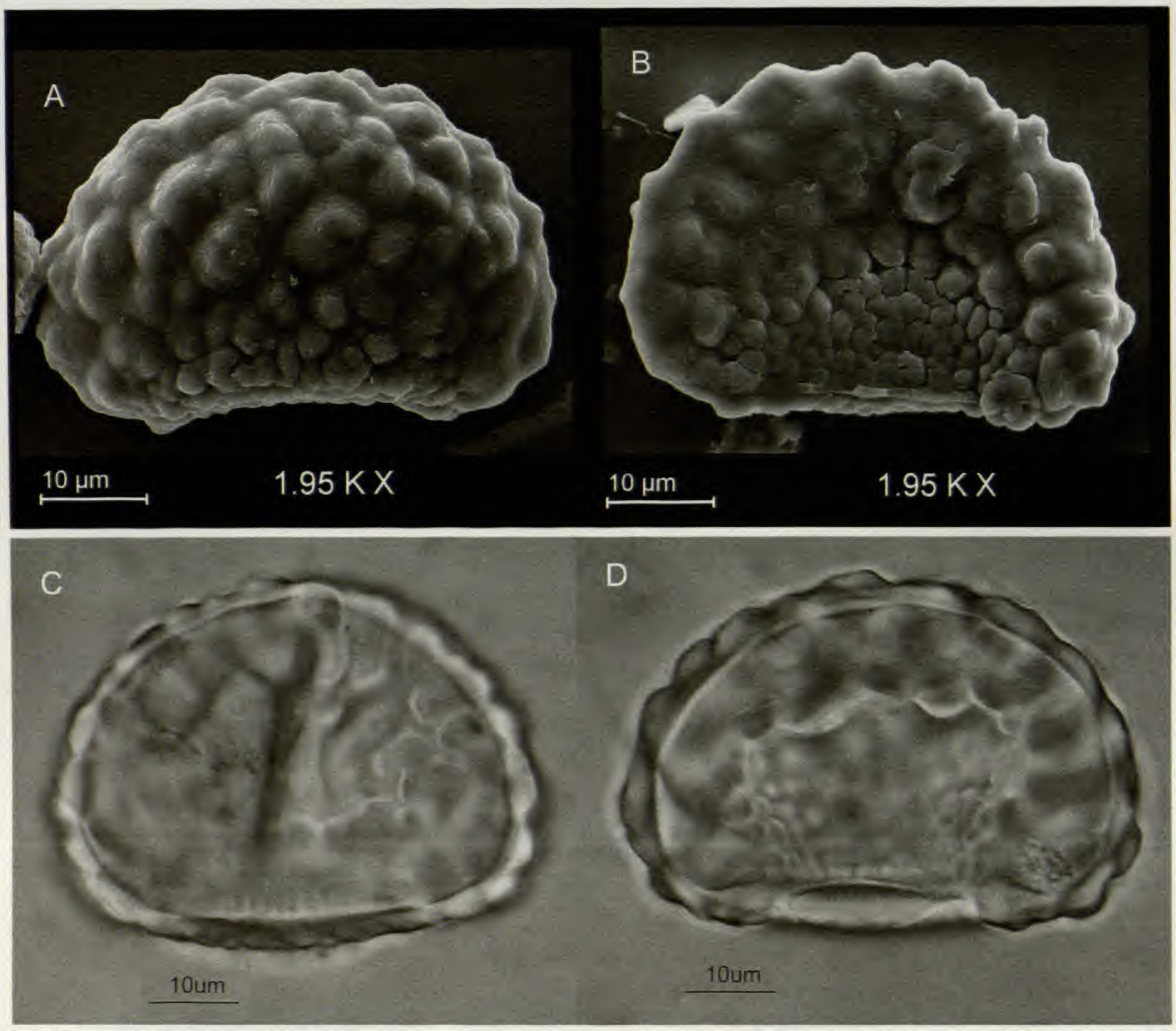

FIG. 3. A–D. *Serpocaulon* × *sessilipinnum* (*J.M. Chaves et al. 299*, CR). A. Well-developed spore in lateral view (Scanning Microscopy). B. Collapsed spore in lateral view (Scanning Microscopy). C. Well-developed spore in front view (Transmission Microscopy). D. Collapsed spore in lateral view (Transmission Microscopy). Photos by Valentina Ramírez-Valencia (April 29, 2013).

(vs. pinnatisect throughout) blade divisions, relatively broader (0.5–1.7 cm vs. 0.5–1.1 cm), deltate to deltate-lanceolate (vs. lanceolate to narrowly deltate) pinnae, relative fewer series (2–3 vs. 1–2) of areoles and fewer series ((1–) 2 vs. 1) of sori, and whitish (vs. yellow) and ellipsoidal to collapsed (vs. ellipsoidal) spores (Fig. 1–2, Table 1).

Besides morphology, the type specimen (*J.M. Chaves et al. 299*, CR, HLDG, MO) was collected among populations of both *S. fraxinifolium* (*J.M. Chaves et al. 297*, CR, HLDG, MO) and *S. ptilorhizon* (*J.M. Chaves et al. 298*, CR, HLDG, MO), what also suggests a hybrid origin involving these two species.

Spore abortion is a key character that provides evidence of hybridization (Moran, 2004), and it has been found in many hybrids in different genera such as *Adiantum* (Moran and Watkins, 2002; Rojas-Alvarado and Herrera-Martínez, 2012), *Asplenium* (Rojas-Alvarado and Chaves-Fallas 2010), and *Polystichum* (Barrington 1985). However, some hybrids can also present

normal spores, as documented by (Ramírez-Valencia et al., 2013, Schwartsburd and Smith, 2013) for *Serpocaulon*. This seems to be the case for the new hybrid here described, which has whitish-translucent spores, but without any content inside (Fig. 3).

Acknowledgments

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Shorter Notes

First Record of *Serpocaulon lasiopus* (Polypodiaceae) from Mesoamerica.—*Serpocaulon* was recently segregated from *Polypodium* by Alan Smith and colleagues (Taxon 55:919–930. 2006). The genus is entirely Neotropical or subtropical in distribution, with 40 species occurring only in South America. One of its species is *S. lasiopus* (Klotzsch) A.R. Sm. (basionym: *Polypodium lasiopus* Klotzsch, Linnaea 20:393. 1847.), whose geographic distribution according to Smith et al. (2006) is Venezuela, Ecuador, Peru, and Bolivia. Specimens of S. *lasiopus* not seen by Smith et al. (2006) were also recently found in Colombia (Idárraga-Piedrahits et al., Fl. Antioquia: Cat. 2:9–939. 2011.), Argentina (A. Paul *pers. com.*, 2013), Haiti (A. Paul *pers. com.*, 2013), and the Dominican Republic (TROPICOS). The species was not treated in *Flora Mesoamericana* (Davidse et al., Fl. Mesoamerica: Vol. 1. 1995), nor has it been reported in the more recent fern volume for the *Flora of Nicaragua* (Stevens et al., Tomo IV, Helechos Flora Nicaragua. 2009).

In 2012 during an epiphyte survey in Cusuco National Park, Honduras (15° 32′ 31″ N and 88° 15′ 49″ W), as part of the first author's Ph.D. research, two small populations of *S. lasiopus* were found in moist broad-leaved mountain forest in the Sierra de Merendón west of San Pedro Sula. The specimens were identified by Alan R. Smith (UC). The first population of S. *lasiopus* was collected 35 m above ground on a large mature tree of *Liquidambar styraciflua* L. (1903 m). The second population was collected at 18 m above ground on a large mature tree of *Ilex pallida* Standl (1877 m). Both populations consisted of fertile individuals but with less than 10 individuals on each tree. The specimens were deposited at Trinity College Dublin (TCD) with one duplicate at UC. Another duplicate will also be transferred to the herbarium in UNAH (TEFH). The specimens are the first record of *S. lasiopus* in Mesoamerica. That *S. lasiopus* has not been previously reported from Mesoamerica is probably because, at least in Honduras, it grows as a canopy epiphyte, making it difficult to detect. Also, there has been little research on epiphytes in Honduras.

In *Flora Mesoamericana, S. lasiopus* falls within *Polypodium loriceum* Group 2 (Davidse et al., Fl. Mesoamerica: Vol. 1. 1995), which was grouped by Smith et al. (2006) within the *P. loriceum, P. fraxinifolium,* and allies Group 7. This group is distinctive by long-creeping rhizomes that bear partially or entirely clathrate, brownish or blackish scales. According to *Flora Mesoamericana* and *Flora of Nicaragua, S. lasiopus* would key out to the *P. dissimile* and/or *P. wagneri* section. What distinguishes *S. lasiopus* from these species are characters of venation, blade dissection, and indument (septate scales/hairs). This species can be distinguished from other *Serpocaulon* species in *Flora Mesoamericana* by having moderately to dense indument on both sides of the pinna consisting of 4–7-celled hairs and rhizome scales 0.4–0.6 cm long (as opposed to 0.6–1.1 cm in the case of *S. dissimile* (L.) A.R. Sm. and 0.2–0.3 cm in the case of *S. wagneri* (Mett.) A.R. Sm.).

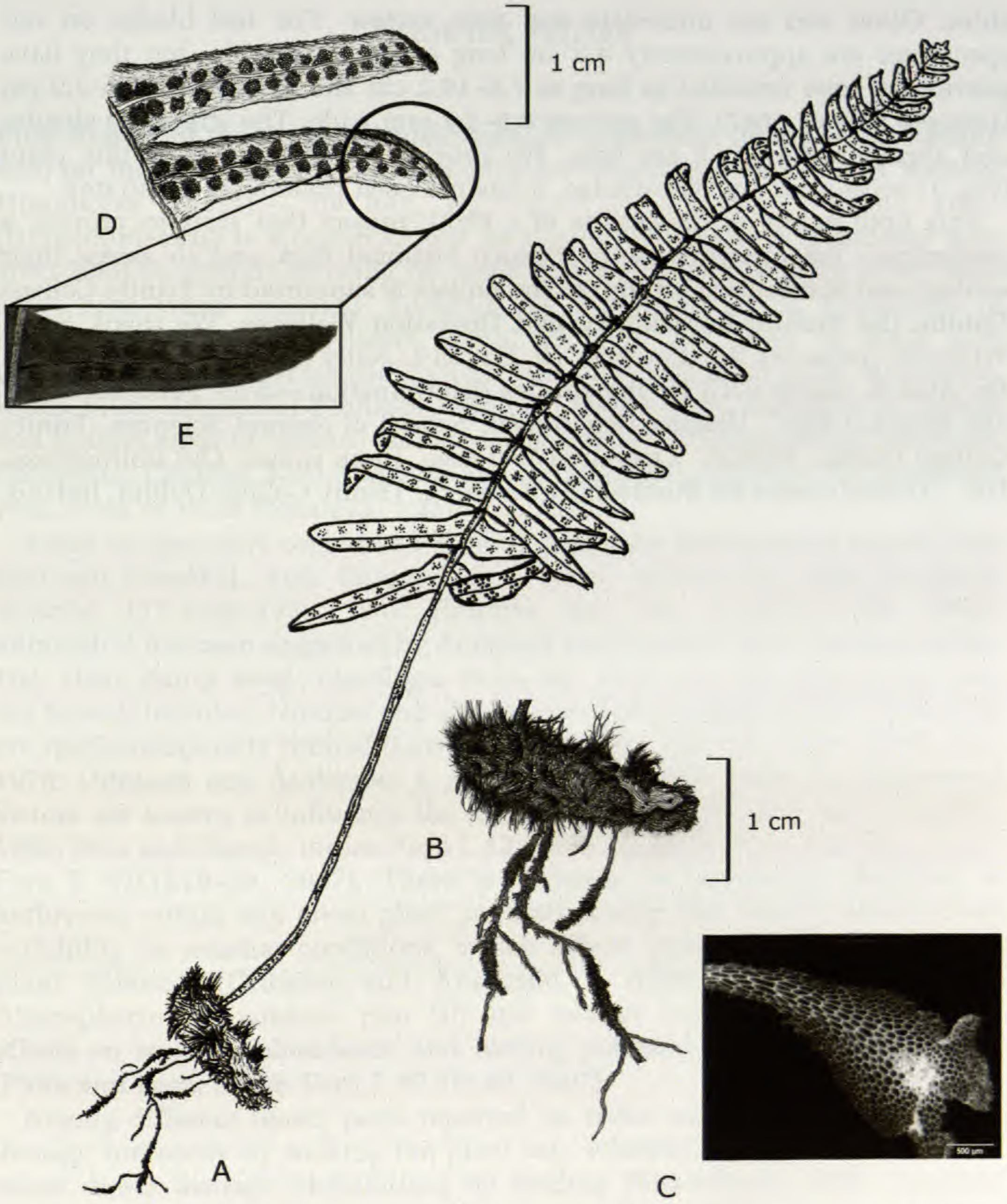

FIG. 1. A–E *Serpocaulon lasiopus*. A. Habit. B. Rhizome. C. Detail of the rhizome scale. D. Abaxial surface detail of pinna with sori. E. Indument on pinna.

The stems of *S. lasiopus* are creeping, thick, fleshy, and fragile, scales lanceolate, long-acuminate, margin scabrous, shiny, dirty brown and glabrous. Leaves are pinnatifid, sublanceolate, attenuate towards the apex, remote and minutely viscid-pubescent on both sides. The lobes are oblong, obtuse, membranous, approximate, horizontal, and weakly deflexed. Rachis and stipe are generally markedly pale yellow and minutely viscid-pubescent on both

sides. Older sori are uniseriate and pale yellow. The leaf blades on our specimens are approximately 4.5 cm long and 0.6 cm wide, but they have previously been recorded as long as 7.6–10.2 cm and as wide as 3.2–3.9 cm (Linnaea 20:393. 1847). The sori are 0.8–2.2 mm wide. The stipes are slender and approximately 12.5 cm long. We provide an illustration of this plant (Fig. 1) because, to our knowledge, it has not been previously illustrated.

This note is one of the results of a Ph.D. project that aims to provide a preliminary list of epiphytes in Cusuco National Park and to assess their ecology and distribution within it. The project is supported by Trinity College Dublin, the Rufford Foundation, and Operation Wallacea. We thank Sarah Willis for preparing the drawings, Dr. Daniel L. Kelly for his supervision, and Dr. Alan R. Smith (UC) for the species determination.—Sven Peter Batke[1,2,3] and Nicholas Hill[2]. [1]Botany Department, School of Natural Sciences, Trinity College Dublin, Ireland. [2]Operation Wallacea, Hope House, Old Bolingbroke, UK. [3]Trinity Centre for Biodiversity Research, Trinity College Dublin, Ireland.

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Shorter Notes

First Report of the Aphid, *Amphorophora ampullata* (Homoptera: Aphididae) on the Fern, *Hypolepis polypodioides* (Hypolepidaceae) from Western Himalayas (India).—The fern, *Hypolepis polypodioides* (Blume) Hook (Hypolepidaceae) is a common fern throughout the Western Himalayas from 1000-2000 m altitude growing on moist and wet places or along open roadsides near water channels. Globally, *H. polypodioides* is distributed in America, Bhutan, China, Japan, Nepal, Pakistan and Tibet. In India, it is distributed in North-West and Eastern Himalaya (Darjeling hills, Meghalaya, Khasi hills, Arunachal Pradesh, Manipur), North-West Himalaya (Kishtwar and Kathua areas of Jammu) and Himachal Pradesh (Dharamshala, Palampur, Dalhousie, Chamba, Kullu, Manali and Shimla hills) (Khullar, An illustrated Fern Flora of West Himalaya, 1:250-253. 1994).

Ferns are generally considered difficult plants for herbivores to exploit (Soo Hoo and Fraenkel, Ann. Entomol. Soc. Amer. 57:788–790. 1964; Kaplanis, Science. 157:1436–1438. 1967; Hendrix, Am. Nat. 115(2):171–196. 1980), although it has been suggested by Auerback and Hendrix (Ecol. Entomol. 5:99–104. 1980; Balick *et al.*, Oecologia 35:55–89. 1978) that this assumption may not be well founded. Number and abundance of phytophagous insects of a host are spatio-temporally limited (Lawton, Symp. Roy. Ent. Soc. Lond. 9:105–125. 1978; Ottosson and Anderson, J. Anim. Ecol. 52:397. 1983). Environmental factors are known to influence the herbivore guilds of a host fern (Hendrix, 1980; Bera and Ghorai, Indian Fern J. 12:168–172. 1995; Patra and Bera, Amer. Fern J. 97(1):19–29. 2007). There is variation in population densities of herbivores within any given plant population over time due to seasonal and variability in weather conditions, which affects insect and plant growth or plant defences (Ottosson and Anderson, J. Anim. Ecol. 52:397. 1983). Atmospheric temperature, rain fall and relative humidity have regulatory effects on seasonal abundance and feeding potential of fern feeding insects (Patra and Bera, Amer. Fern J. 97:19–29. 2007).

Among different insect pests reported on ferns, sucking pests cause more damage indirectly by sucking the plant sap, whereas beetles and caterpillars cause direct damage (defoliation) by feeding (Birkenhead, 1892; Hendrix, Amer. Nat. 115(2):171–196. 1980). To our knowledge there are no published reports of herbivory by insects on *H. polypodioides*. However, during frequent visits to the IHBT Fernery to study the impact of insect on different ferns, an infestation of the aphid, *Amphorophora ampullata* Buckton on *H. polypodioides* was noticed. To study the incidence of *A. ampullata* in the experimental plot (20 sq. m), 20 fronds were randomly selected for sampling. The number of *A. ampullata* (nymphs and adults) was counted on five randomly selected pinnae/frond and represented as number of aphids/pinna. Infestation of *A. ampullata* on *H. polypodioides* was first observed during last week of August 2012 (7.3 aphids/pinna). The infestation increased gradually

FIG. 1. *Amphorophora ampullata* congregating and sucking the sap from *Hypolepis polypodioides*.

during last week of September and October (14.5 and 17.3 aphids/pinna respectively) and peaked during the last week of November of 2012 (24.8 aphids/pinna) which may due to high temperature (28–30 °C) and humidity (65–75%), later the infestation was decreased in the month of December (5.9 aphids/pinna) and January 2013 (0.10 aphids/pinna) due to low temperature (4–10 °C). The fern aphid, *A. ampullata* is greenish to brown, bigger in size as compared to other aphids. The alate male body is about 2.71 mm length, 0.99 mm width and antennae (n=1) are longer than the body (3.42 mm) (Raychaudhuri *et al.*, Ins. Matsum. n.s., 20:1–42. 1980). In the present study, the alate male body is about 2.91 mm length, 1.03 mm width and antennae are longer than the body (3.45 mm); whereas, the alate female body is about 4.15 mm length, 2.04 mm width and antennae are 5.38 mm long (n=10).

Both nymphs and adults stages of aphid are responsible for causing damage to *H. polypodioides* and were found congregating the under (lower) surface of fronds (Fig. 1). Apparently the aphids injure the plant by piercing and sucking the sap, which results in yellowing, loss of vigour, drying, and dropping of fronds. In severe infestations, entire plants start drying and wilting.

In India, *A. ampullata* is distributed in Arunachal Pradesh, Himachal Pradesh, Meghalaya, Sikkim and West Bengal on different host plants other than *H. polypodioides* (Raychaudhuri *et al.*, Ins. Matsum. n.s., 20:1–42. 1980),

but to our knowledge this is the first report of *A. ampullata* on *H. polypodioides* from Himachal Pradesh. Lozzia and Binazzi (Bollettino di Zoologia Agraria e di Bachicoltura, 33(2):125–131. 2001) reported information related to the geographical distribution, ecology, biology, and morphology of both apterous and alate viviparous females of *A. ampullata.*

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Shorter Notes

***Lygodium japonicum* (Thunberg) Swartz in the Piedmont of Georgia.**—Recent collections have documented the spontaneous occurrence of this invasive nonnative species in the Piedmont of Georgia, which has previously been known, outside of cultivation, only from the Atlantic Coastal Plain. *Lygodium japonicum* (Thunb.) Sw. (Japanese Climbing Fern) is native to open forests of temperate, subtropical, and tropical regions of eastern and northeastern Asia, the East Indies, and northern Australia (Leichty et al. Castanea 76:293–299. 2011). Wherry (The Southern Fern Guide. Doubleday, New York. 1964) described it as "escaped from cultivation over much of [the Southeast]" and stated that *L. japonicum* has been "known in North Carolina since 1900 and in Florida since 1932." Its range now also includes Alabama, Arkansas, Georgia, Louisiana, Mississippi, South Carolina, and Texas (Nauman. *Lygodium*. In: Flora of North America, Oxford University Press, New York. 1993; Weakley. Flora of the Southern and Mid-Atlantic States. The University of North Carolina Herbarium, Chapel Hill. Draft of 30 November 2012.).

Because it can smother other vegetation, *L. japonicum* poses a threat to native plants in pine and hardwood forest communities of the Southeast, with estimates of its present coverage ranging up to 74,000 hectares (Bohn et al. Ecological Restoration 29:346–356. 2011). A number of traits contribute to its invasive potential, including small, highly dispersible spores and a capacity for intragametophytic self-fertilization (Lott et al. Amer. J. Bot. 90:1144–1152. 2003). More recently it has been documented to invade species-rich pine savannas, where its underground rhizomes allow it to survive fires and quickly increase in abundance to the detriment of native species (Leichty et al. 2011). It is also reported to increase the probability of crown fires as the dried winter foliage carries fire high into the canopy of infested trees (Gray et al. 2009). Leichty et al. (2011) concluded that "current prescribed fire techniques alone appear insufficient to limit or even slow *L. japonicum* invasion of pine savannas" and suggested that use of herbicides might be required.

McVaugh and Pyron (Ferns of Georgia. University of Georgia Press, Athens. 1951) noted that, in Georgia, *L. japonicum* often escapes to roadsides, banks of ditches and streams, and sometimes alluvial woodlands, where it occasionally becomes well-established and almost weedy. Since then its invasive potential has become better appreciated, as it has dramatically increased its range across Georgia and the Southeast, being labeled a Category I invasive species by both the Georgia Exotic Pest Plant Council (Gray et al. Invasive Plants to Watch for in Georgia. The University of Georgia Center for Invasive Species and Ecosystem Health, Tifton, GA, BW-2009-100. 55 pages. 2009) and the Florida Exotic Pest Plant Council (Hutchinson and Langeland. Amer. Fern J. 100:57–66. 2010).

In Georgia *L. japonicum* was first collected in Lowndes County by J. R. Stokes (*s.n.*, along stream banks, Valdosta. 2 Feb 1926.). [All cited collections

are housed at GA.] Following the ecoregions of Georgia as delimited by Edwards et al. (The Natural Communities of Georgia. University of Georgia Press, Athens. 2013), this site is in the Upper Coastal Plain, where all collections prior to 1960 originated. Since then another 20 localities have been added, based on a survey of the 41 specimens at GA. The first collection from the Lower Coastal Plain was not made until 1963, with 10 subsequent collections from this region. For the Piedmont the species is only known from four wild specimens, all taken since 1999. Supporting the view of early infestation in extreme southeastern Georgia is the fact that only Decatur, Seminole, and Marion Counties, all in the Upper Coastal Plain, are reported to have more than 400 hectares occupied by *L. japonicum* (Miller et al. Maps of occupation and estimates of acres covered by nonnative invasive plants in southern forests using SRF FIA data posted on March 15, 2008. Viewed at http://www.invasive.org/fiamaps/1 April 2013.).

This pattern of invasion, proceeding from the Upper Coastal Plain to the Lower Coastal Plain and finally to the Piedmont is reinforced by published county distribution maps. McVaugh and Pyron (1951) showed the occurrence of *L. japonicum* in six counties, all in the Upper Coastal Plain. In their update of this book, Snyder and Bruce (Field Guide to the Ferns of Georgia. University of Georgia Press, Athens. 1986) noted its occurrence in 26 counties. Of these, 20 were in the Upper Coastal Plain; five, the Lower Coastal Plain; and one, the Piedmont. The Piedmont record from Clarke County, however, was apparently based on cultivated material. The two collections in question were by Maguire (*s.n.* In cultivation, Athens. 1928) and by Jones (22614. Weed in azalea bed—apparently it came in from Mobile, where it is often a weed. 1975).

More recent collections documenting spontaneous records from the Piedmont of Georgia come from Harris (Sweeney 536. 1999.), Fulton (Reynolds 74. 2006.), Cobb (Zomlefer 1107. 2006.), and Oconee Counties (R. Wyatt 1879. 2012.). All of these collections noted that the plants were rare, usually represented by a single individual. In our own experience *L. japonicum* came in on soil disturbed by the construction of wooden steps built in 2007. The single plant, first noticed in 2009, grew for about three years before becoming large enough to be recognizable. Rabbits regularly ate the young growth until we placed a screen wire cage around it and allowed the plant to mature. A soil sample was taken for analysis and revealed nothing unusual about the substrate: like most Piedmont soils, the pH was low (5.2) and levels of phosphorus, potassium, and calcium were relatively low (0.56, 8.3, and 29 g/m^2, respectively). Nevertheless, most authors, such as Wherry (1964), Snyder and Bruce (1986), and Nauman (1993), have cited *L. japonicum* as preferring circumneutral soil.

There is reason to expect that Japanese Climbing Fern may continue to extend its range and become a major problem in the Piedmont of the Southeast, especially if there is further warming of the climate. The widespread use of pine needle mulch, often transported from the Coastal Plain to the Piedmont, also may hasten its spread as described by Gray et al. (2009) and Hutchinson and Langeland (2010). On the other hand, it is possible that *L. japonicum* will

not be able to maintain itself indefinitely. Larry Mellichamp (personal communication) has observed it over the past 40 years in the University of North Carolina at Charlotte Botanical Garden, where it has come in as spores to pitcher plant bogs and other areas that receive supplemental water. He doubts, however, that it can survive long-term in the zonal vegetation of the Piedmont because of unevenness of rainfall and low winter temperatures.

The plant in Oconee County, however, was able to persist despite temperatures as low as −10°C (14 January 2011: Data from the Georgia Automated Environmental Monitoring Network, Watkinsville site, accessed at www.georgiaweather.net). Historically, record low temperatures for the Piedmont of Georgia have been much colder than this, with −23°C having been recorded in Atlanta in February of 1899. Most of the record low temperatures, however, occurred more than 30 years ago (accessed at www.intellicast.com). In our judgment, it is likely that *L. japonicum* will continue to spread into the Piedmont and displace native vegetation. Land managers need to be alert to its presence and eradicate the plants, if possible, before they have a chance to become well established.

We thank John T. Mickel for consultation on the identity of immature material of *Lygodium*, Brenda L. Wichmann and Wendy B. Zomlefer for help with specimens in the GA Herbarium, Carl Rothfels and two anonymous reviewers for editorial advice, and Larry Mellichamp for sharing his observations of *L. japonicum* over a 40-year period.—ROBERT WYATT[1] AND GRAHAM E. WYATT[2]. [1]Georgia Museum of Natural History, University of Georgia, Athens, GA 30602. [2]Department of Plant Biology, University of Georgia, Athens, GA 30602.

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Review

Ferns and Fern Allies of Taiwan, by R. Knapp. KBCC Press & Yuan-Liou Publishing Co., Ltd., Taipei. 2011. 1052 pp. Hardback. Price: $100.00 (available at fernsandfernalliesoftaiwan.blogspot.tw and www.mbgpress.info). ISBN 978-986-87098-0-5.

Taiwan and its surrounding islets encompass about 35,800 square kilometers or 13,822 square miles, an area just about 1,400 square miles larger than the state of Maryland in the United States. As "the garden of ferns", Taiwan has been well known for its rich diversity of ferns and lycophytes that flourish in a spectacular variety of habitats ranging from tropical mangrove swamps to rain and cloud forests, to seasonal dry forests, and to alpine meadows.

Exploration of the diversity of ferns and lycophytes in Taiwan dates back to the 19th century. Henry (1896; *A List of Plants from Formosa* in Trans. Asiat. Soc. Japan. 24:suppl. 1–118) published the first relatively comprehensive list of plants of Taiwan including 149 species of ferns and lycophytes in 34 genera. Subsequently, these numbers were revised by various authors: Hayata (1914–1919; *Icones plantarum formosanarum*) reported 537 species in 75 genera; Daigobo et al. (1975) documented 540 species in 172 genera in *Flora of Taiwan*; Kuo (1985; Taiwania:30:5–99) recognized 617 taxa excluding hybrids in 107 genera; Chiou et al. (1994) recorded 597 taxa in *Flora of Taiwan*, ed. 2. After 17 years, the number of species of ferns and lycophytes in Taiwan was increased to 729 by Knapp (2011), a German engineer who works for a technical company in Taiwan.

Knapp's *Ferns and Fern Allies of Taiwan* is an illustrated introduction to the ferns and lycophytes of Taiwan including the islands of Penghu, Kinmen, Matsu, and neighboring islets. The author adopts Kuo's (1985) vegetative zones by dividing Taiwan into eight well-defined vegetative zones that encompass a diversity of habitats from the western alluvial plains to the rugged mountains in the east. Many terms in the glossary are illustrated with photographs. Distribution and frequency data for all taxa are a culmination of years of fieldwork, extensive survey of herbaria and literature collections, and diligent exchange of information with local and foreign specialists. An introduction to the few invasive and naturalized species, as well as background information for critical and rare taxa, some genera, and nomenclatural changes is also furnished. The comprehensive volume includes descriptions of both previously published and unpublished taxa. The taxonomic system used follows the morphology-driven concept of Kramer & Green (1990) though newer systems, e.g., that of Smith et al. (2006), are sometimes discussed. Dichotomous keys provide for the precise identification of families, genera and lower taxa; each key is followed by a set of photographs portraying the entire plant and relevant details introduced in the text. In almost all cases, distinguishing characters of a particular taxon are always well shown with color close-ups, separating this

book from many general ones. All delineations are accompanied by the scientific binomial, the Chinese name, and the pinyin transliteration. In the key, the stressed syllables in the Latin names are underlined, which is reminiscent of Werner Rothmaler's *Exkursionsflora von Deutschland* and is much appreciated. Every taxon is illustrated with at least one photograph, thus facilitating the accurate identification of every currently known species, subspecies, and hybrid in Taiwan. Even very rarely and poorly documented species are discussed with several photos, some of which are a full page in size, bringing the total number of color images to over 4,700, some of which are of types of species or infraspecific taxa making this book particularly valuable for not only fern lovers but also professional taxonomists! A thorough index includes numerous synonyms, misapplied names, and alternative taxonomic combinations.

As with every book published, Knapp's (2011) book could have been out of date right after it was submitted to the press. Readers, however, will be glad to see that a supplemental volume to his 2011 book – *Ferns and Fern Allies of Taiwan – Supplement* (Taipei: KBCC Press. 212 pp. Paperback. Price: $35.00. ISBN 978-986-87098-1-2) was published in March 2013 with 560 new color images. The *Supplement* contains some additions and corrections, bringing the total number of species of ferns and lycophytes in Taiwan to 747. Many field images used in the book are available for free at https://picasaweb.google.com/116136418529949606360.

In China's current 32 provinces, Taiwan has 4,383 species of vascular plants and is the seventh most species-rich province for vascular plants (the first six are Yunnan, Sichuan, Guangxi, Xizang, Guizhou, and Guangdong; Wu et al., 1994–2013). When only species of ferns and lycophytes are counted, Taiwan is the fourth richest province in China (the first three are Yunnan, Sichuan, and Guizhou; Lin et al., 2013). Interestingly, in terms of species numbers of ferns and lycophytes per square mile, Taiwan has not only the highest fern diversity of any province in China but also is one of the most fern-diverse regions in the world, in comparison with, e.g., 509 species and infraspecific taxa of ferns and lycophytes in 70 genera in the whole of North America, north of Mexico (*Flora of North America* Editorial Committee, 1993).

With Knapp's (2011, 2013) publications this rich flora of ferns and lycophytes in Taiwan is now well documented and is definitely one more big step closer to the final version. With the new version of *Flora of China* vol. 2–3 (pteridophytes) published (Lin et al., 2013) and more and more molecular data accumulated, further revision of the fern and lycophyte flora of Taiwan is expected, with new familial and generic delimitations incorporated. We look forward to seeing updates of Knapp's work on the flora of ferns and lycophytes of Taiwan in the near future.—Li-Bing Zhang, Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166-0299, U.S.A. and Chengdu Institute of Biology, Chinese Academy of Sciences, P.O. Box 416, Chengdu, Sichuan 610041, P. R. China; Hai He, College of Life Sciences, Chongqing Normal University, Shapingba, Chongqing 400047, P. R. China.

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Patterns of North American Fern and Lycophyte Richness at Three Taxonomic Levels

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ABSTRACT.—North American monilophyte (fern) and lycophyte richness patterns are examined at three taxonomic levels (species, genus, and family). We determine: (1) if fern richness patterns are associated with water and energy variables that are predicted by the productivity-diversity hypothesis and (2) whether the pattern or strength of the relationship varies with taxonomic level. We present species richness maps for individual families of ferns and lycophytes allowing us to identify taxa with unique distributional patterns and taxa with patterns comparable to ferns in general. To accomplish these goals, we use data from the Flora of North America project for continental North America north of Mexico plus Greenland. We construct 479 GIS fern species range maps and tabulate fern and lycophyte richness in a gridded map with 2500km^2 squares. We perform regressions of fern richness on water and energy climate variables (with squares as data points) in order to identify which variables most influence fern richness. We find that fern richness correlates with water and energy variables in ways consistent with the productivity-diversity hypothesis. A multiple regression model that includes mean annual temperature (MAT) and annual rainfall (RAN) explains 78.1% of the variation in fern family richness. The relationship between fern *family* richness and climate is stronger than the relationship between fern *species* richness and climate.

KEY WORDS.—fern, lycophyte, GIS, richness, taxonomic richness, North America, productivity-diversity hypothesis

Scientists have long been aware of latitudinal richness patterns, where richness tends to decrease from low to high latitudes in a wide array of organisms (Wallace, 1878). Plants are believed to play a central role in governing broad latitudinal gradients in species richness (Kreft and Jetz, 2007; Kier *et al.*, 2005; and references therein) because they constitute the primary trophic level and their physiological requirements are proximally affected by environmental factors related to climate, primarily water and energy availability (Currie and Paquin, 1987; Field *et al.*, 2005; O'Brien, 1993; O'Brien, 2006). The important effect of climatic variables on richness is supported by recently developed empirical climate models in which factors related to water and energy availability are highly successful in predicting woody plant species richness (Field *et al.*, 2005).

A number of similar hypotheses for plant latitudinal diversity gradients hold that gradients in productivity drive gradients in diversity, where higher productivity leads to greater diversity (Field *et al.*, 2009; Hawkins *et al.*, 2003),

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which we refer to as the productivity-diversity hypothesis. In this study we examine the relationship between climate and monilophyte (fern) richness at three taxonomic levels, in order to test the productivity-diversity hypothesis. We use the Smith *et al.*, (2006) fern taxonomic classification system. First, we test a number of regression models that employ linear combinations of simple climate variables to determine whether these variables predict fern richness. We also test whether one particular richness-climate model, the interim general model I (IGM I), accurately predicts fern richness. We employ the IGM I because it successfully predicts richness gradients in other plant groups and it has a plausible theoretical basis (O'Brien, 2006).

A theoretical basis for linking the generation of plant diversity with climate factors is provided in water-energy dynamics theory (WED; O'Brien, 1998; O'Brien, 2006), which can be included among productivity-diversity hypotheses. WED states that plant biological activity is related to *liquid* water and energy availability, which foster higher productivity, and finally lead to greater biological richness. Climate factors associated with greater plant productivity also lead to greater rates of molecular evolution (see Rohde, 1992; Wright *et al.*, 2003; Wright *et al.*, 2006) or greater amounts of biological activity in general (generation time, faster growth, more individuals). Higher rates of molecular evolution lead to more population divergence (Martin and Mckay, 2004), which eventually leads to greater numbers of species. The WED hypothesis is related to the evolutionary rates hypothesis (ERH) which predicts that diversity will be associated with conditions that cause higher rates of molecular evolution (Evans and Gaston, 2005). For plants, regions with higher (or optimal) temperatures and more liquid water are likely to be associated with higher rates of molecular evolution and higher rates of productivity.

The interim general model (IGM I) is a climate model designed to predict plant richness based on the principles of WED theory (Field *et al.*, 2005) taking the following form:

$$Richness = \beta_1 RAN + \beta_2 PETmin - \beta_3 PETmin^2 + \beta_0$$

Where PETmin is the minimum monthly potential evapotranspiration and RAN is annual rainfall. Before each term are empirically fitted coefficients. Rainfall is included to reflect that plants require the liquid form of water. PET (potential evapotranspiration) is an index of energy; its relationship with richness is expected to be nonlinear. WED predicts greatest richness where water and energy climate conditions are most favorable for plant productivity and biological activity, while the IGM provides a hypothesis for, and quantification of, what these conditions would be.

For many organisms, species richness correlates with water and energy variables in a similar way as taxonomic richness at higher levels. We know of no reason *a priori* why ferns would be unusual in this regard. Family richness, additionally, may serve as a proxy of species richness patterns (Balmford *et al.*, 1996; Francis and Currie, 2003; Gaston and Blackburn, 1995; Qian and

Ricklefs, 2004) if for some reason doubt is placed on the completeness of species level data. To test whether richness-climate relationships are similar across taxonomic levels, all climate models are examined with respect to species, genus and family richness.

Ferns and lycophytes.—As separate clades of vascular plants, ferns and lycophytes may be compared to seed plants to assess the generality of richness patterns and richness-climate relationships. Ferns and lycophytes share with other plants the same basic requirements of water, light, and nutrients that have been shown to be key factors in determining plant species distribution (Hengeveld, 1990; Holdridge, 1947; O'Brien, 1998; Salisbury, 1926; Woodward, 1987) and, thus, we expect them to have similar richness-climate relationships as other plant groups. However, ferns and lycophytes differ from other plants in key morphological and life-history traits. First, ferns and lycophytes have a unique life cycle that includes external fertilization during the gametophytic stage, requiring environmental liquid water (Raven *et al.*, 1992). This reliance on external water during reproduction may impose limitations on the geographic distribution of fern species and gradients in species richness (Given, 1993; and references therein), although the existence of xeric adapted ferns demonstrates that this limitation is surmountable. Ferns have evolved a variety of aridity adaptations (Hietz, 2010; Kessler and Siorak, 2007). However, a recent analysis of global pteridophyte (ferns and lycophytes) richness patterns by Kreft *et al.* (2010) found that, relative to seed plants, fern and lycophyte richness more strongly correlates with liquid water regimes. Fern and lycophyte richness dropped more rapidly than seed plant richness when moving toward drier climates.

Second, fern and lycophyte spores are readily dispersed over extremely long distances; thus their distributions are less likely to be limited by dispersal barriers and more likely to be limited by the ability to adapt and persist under climates outside their current range (Barrington, 1993). Fern and lycophyte species distribution could be expected to match the climatic conditions that allow a species to become established and persist, or at least more so than other groups. In examining fern and lycophyte distribution the effect of dispersal on distributional patterns can be evaluated by comparison with groups with more constrained dispersal (Barrington, 1993). In addition, fern richness patterns have been studied on several continents allowing us to compare our results to other regions of the world. Fern richness patterns (or pteridophyte richness patterns) have been studied at regional scales in Northeast Iberia (Pausas and Saez, 2000), Japan (Guo *et al.*, 2003) and Argentina (Ponce *et al.*, 2002); along elevational gradients (Bhattarai *et al.*, 2004; Hemp, 2002; Watkins *et al.*, 2006), and at continental scales including Africa (Aldasoro *et al.*, 2004), Australia (Bickford and Laffan, 2006) and Europe (Birks, 1976). Kreft *et al.* (2010) analyzed patterns of global pteridophyte diversity.

The primary objective of this study is to characterize the geography of North American fern and lycophyte richness (north of Mexico). The second goal is to explore potential climatic controls of observed richness patterns. Ferns and

TABLE 1. Summary of environmental variables used in this study.

Abbreviation	Environmental variable	Units	Source
AET	Mean annual actual evapotranspiration	mm	Global Climate Database[1]
PETmin	Minimum month potential evapotranspiration	mm	Global Climate Database
PET	Mean annual potential evapotranspiration	mm	Global Climate Database
RAN	Annual rainfall	mm	Global Climate Database
MAT	Mean annual temperature	°C	Worldclim[2]
Bio4	Temperature seasonality (st. dev. of monthly temperature)	°C	Worldclim
Bio13	Precipitation of the wettest month	mm	Worldclim
Bio15	Precipitation seasonality (st. dev. of monthly precipitation)	mm	Worldclim
Bio16	Precipitation of driest quarter	mm	Worldclim

[1] (Leemans and Cramer, 1991).
[2] (Hijmans *et al.*, 2004).

lycophytes are mapped and analyzed separately and most analyses apply to ferns. In order to accomplish these goals we map geographical patterns of fern richness at family, genus, and species levels and compare these patterns to the patterns found in other groups of organisms. Second, we examine the relationships between richness and climate variables that are known to be related to plant productivity (Table 1; Stephenson, 1998) at the three taxonomic levels. Among the regression models tested is the interim general model I (IGM I; Field *et al.*, 2005; here fitted with coefficients matched to fern richness). Third, we map and compare the species richness patterns of individual fern and lycophyte families. We identify taxa with unusual distributional patterns and taxa with patterns comparable to ferns in general.

MATERIALS AND METHODS

Study system.—The North American ferns and lycophytes are two divisions of vascular plants. Ferns and lycophytes together (pteridophytes) are a paraphyletic grouping, but each taxon alone is monophyletic (Pryer *et al.*, 2004). The majority of species in this study are ferns (387 out of 479). Fern and lycophyte distribution data for this study derive from the Flora of North America Vol. 2 (FNA editorial committee, 1993). We used the more recent Smith *et al.*, (2006) fern taxonomic classification system for reported analyses and maps instead of the FNA taxonomic classification system. Results are similar regardless which taxonomic system is chosen. The study region contains 23 families, 67 genera, and 387 species of ferns and 3 families, 9 genera, and 92 species of lycophytes. We treat the eighty-five hybrids, varieties, and subspecies as species in the same manner as Tryon (1972). The use of sub-specific designations in ferns allow pteridologists to provide information about their beliefs with respect to the amount of divergence between populations - certain populations can be designated as subspecies or varieties - putatively in different stages of divergence, which would otherwise

be considered separate species by some taxonomists (Tryon, 1969). It is common for fern taxonomists to use subspecific designations to distinguish similar but distinct non-intergrading taxa (Hickey *et al.*, 1989; Yatskievych and Moran, 1989). Regardless, many of these types are geographical subspecies and so the species would not be counted twice in any one location. Richness patterns are largely unchanged when hybrids are excluded and subspecies or varieties are considered as single species.

Range maps.—We used published non-GIS fern range maps from the Flora of North America North of Mexico Vol. 2, Pteridophytes and Gymnosperms (FNA editorial committee, 1993) to produce geo-referenced range map polygons for GIS analysis. Image files were obtained online at http://www.fna.org/. The image files were used as a template on which to produce 479 polygon shape files in the ArcView 9.1 GIS software (Esri, 2005). The non-GIS range maps were produced by the author of each taxon treatment in the Flora by hand drawing shaded regions from herbarium records (see Flora of North America volume 2).

Richness maps.—All analyses were performed on a North American Lambert conformal conic projection with standard parallels at 20°and 60°N latitude and the central meridian at 96°W. A polygon shapefile layer with a grid of 50 km × 50 km (2,500 km^2) was created over North America using Hawth's Analysis Tools for ArcGIS (Beyer, 2004) and used as a base-map. All maps were generated with this grid with a total of 8,760 squares. Regression analyses were applied to a sub-sample of this grid, 88 squares, in order to account for the effect of spatial autocorrelation on the regression results (described below). The Lambert conformal conic projection has low areal distortion in our region of analysis (Bolstad, 2005) and is not expected to affect the results.

Richness in each grid square was determined by summing the number of species range map polygons that overlapped each grid square. This was accomplished using Hawth's tools polygon in polygon analysis (Beyer, 2004). The number of families and genera occurring in each grid square was tabulated similarly.

Climate variables and regression models.—The climate variables tested and their sources are listed in Table 1. Worldclim variables represent climate normals for the period 1950–2000 (Hijmans *et al.*, 2005), while actual evapotranspiration (AET) and potential evapotranspiration (PET) are the normals from 1931–1960 (Leemans and Cramer, 1991). Annual Rainfall (RAN) is calculated as the sum of precipitation of months with a mean temperature greater than zero degrees Celsius. Worldclim climate maps were 2.5 minute resolution. Maps of climate variables were converted to vector point maps when necessary and summarized in each 2,500 km^2 grid square using a spatial join in Arcview 9.1 (Esri, 2005); the average value of the points in the climate layer in each grid square was then determined. Ordinary least-squares (OLS) regressions were performed between richness variables and climate variables with 2,500 km^2 grid squares forming the sample points. Regressions also were performed on an 88 square sub-sample of the total set of 8,760 squares to assess

spatial autocorrelation issues. Regression analyses were performed with the R statistical package (R core development team, 2009).

We present the univariate regressions between each richness level and each climate variable (Table 1) with the highest R-squared values (Table 2). We also perform and present several multiple regressions (Table 2). We performed two, two-variable multiple regressions with the first regression using mean annual temperature (MAT) and AET and the second using MAT and RAN. Finally, we test the interim general model I (IGM I) on our fern richness data (equation above). The models presented in table 2 correspond closely with the best performing models based on Mallow's CP (Mallow, 1973) and a best subsets regression, performed using variables from Table 1. AIC values for each regression model are provided. We report adjusted R-squared values throughout.

Biogeographical patterns are frequently explored with summary statistics without considering geographical pattern (Ruggiero and Hawkins, 2006). Here we present and discuss maps and explicitly evaluate model performances with respect to geography. Mapping residuals of richness-climate regression models allows us to identify specific regions and taxa responsible for model shortcomings, potentially identifying causal factors through spatial association.

Spatial autocorrelation.—In a spatial regression, if residuals are spatially autocorrelated this can potentially mean that the effective sample size is lower than the actual sample size (Fortin and Dale, 2005). As a result, we assess the impacts of spatial autocorrelation by calculating the effective sample size of our data set. Then we use a subsample of grid squares from our full data set (that is even smaller than the effective sample size) to evaluate the robustness of our results.

Equation 5.17 from Fortin and Dale, (2005) allows one to find the effective sample size of a spatial data set for a given spatial autocorrelation ρ between adjacent grid squares:

$$n' \approx n\frac{1-\rho}{1+\rho} = n\Theta$$

Where n is the sample size, n' is the effective sample size, and Θ is the approximate correction factor $\Theta = (1-\rho)/(1+\rho)$. To find the effective sample size of our data set, we first calculated the autocorrelation coefficient ρ between the regression residuals of adjacent squares in a regression using all 8,760 squares. We used residuals from the regression model with fern family richness as a response and MAT, RAN, and Bio15 (precipitation seasonality) as predictors, mapped in Fig. 1D. For this model $\rho = 0.8946$ between adjacent squares. We obtain similar results for the residuals of other regressions presented in the study. Plugging this value into equation 5.17 from Fortin and Dale (2005) yields an effective sample size n' = 487. We conservatively selected only 88 grid squares from our map using a hexagonal sampling grid with points spaced ~500km apart (which is equivalent to a $\rho = 0.98$). Using this conservative subsample, we perform standard OLS regressions in the same fashion as the 8,760 grid values. Equation coefficients and R-squared values were very similar whether we used the subsample, or the full dataset.

TABLE 2. Richness-climate regressions. Listed are seven models, three univariate, and four multiple regressions – one of which is the interim general model (IGM I). Each model was tested with species, genus, and family richness as response variables. R-squared, model AIC, and regression equation coefficients are listed for each model for each response variable.

			Parameter coefficients and significance							
Model	Resp.	adj. R^2	MAT	AET	RAN	PETmin	PETmin2	Bio15	Intercept	AIC
MAT	Fam.	0.676	**0.325**						**7.66**	408.79
	Gen.	0.678	**0.609**						**12.09**	518.27
	Spp.	0.539	**1.23**						**26.02**	693.11
AET	Fam.	0.678		**0.0114**					**3.09**	408.26
	Gen.	0.624		**0.0204**					**3.89**	532.05
	Spp.	0.449		**0.0392**					**10.26**	708.67
RAN	Fam.	0.675			**8.99e-3**				**3.89**	409.02
	Gen.	0.611			**0.0160**				**5.38**	534.91
	Spp.	0.460			**0.0314**				**12.85**	706.96
MAT + AET	Fam.	0.776	**0.186**	**6.55e-3**					**5.04**	377.49
	Gen.	0.747	**0.390**	**0.0103**					**7.97**	498.03
	Spp.	0.570	**0.876**	**0.0164**					**19.44**	687.93
MAT + RAN	Fam.	0.781	**0.188**		**5.19e-3**				**5.49**	375.19
	Gen.	0.748	**0.399**		**7.97e-3**				**8.76**	497.80
	Spp.	0.580	**0.850**		**0.0143**				**20.06**	685.91
IGM I.	Fam.	0.711			**7.67e-3**	**0.15**	−2.02e-3*		**3.69**	400.86
RAN + (PETmin	Gen.	0.665			**0.0123**	**0.273**	−2.57e-3		**5.16**	523.66
- $PETmin^2$)	Spp.	0.470			**0.0268**	0.418	−4.88e-3		**12.39**	707.33
MAT + RAN +	Fam.	0.807	**0.180**		**4.30e-3**			**−0.0413**	**7.74**	365.26
Bio15 or precip.	Gen.	0.762	**0.387**		**6.67e-3**			−0.060*	12.02	493.81
seasonality	Spp.	0.616	**0.809**		9.70e-3*			**−0.21**	**31.55**	678.93

bold $P < 0.001$; * $P < 0.01$.

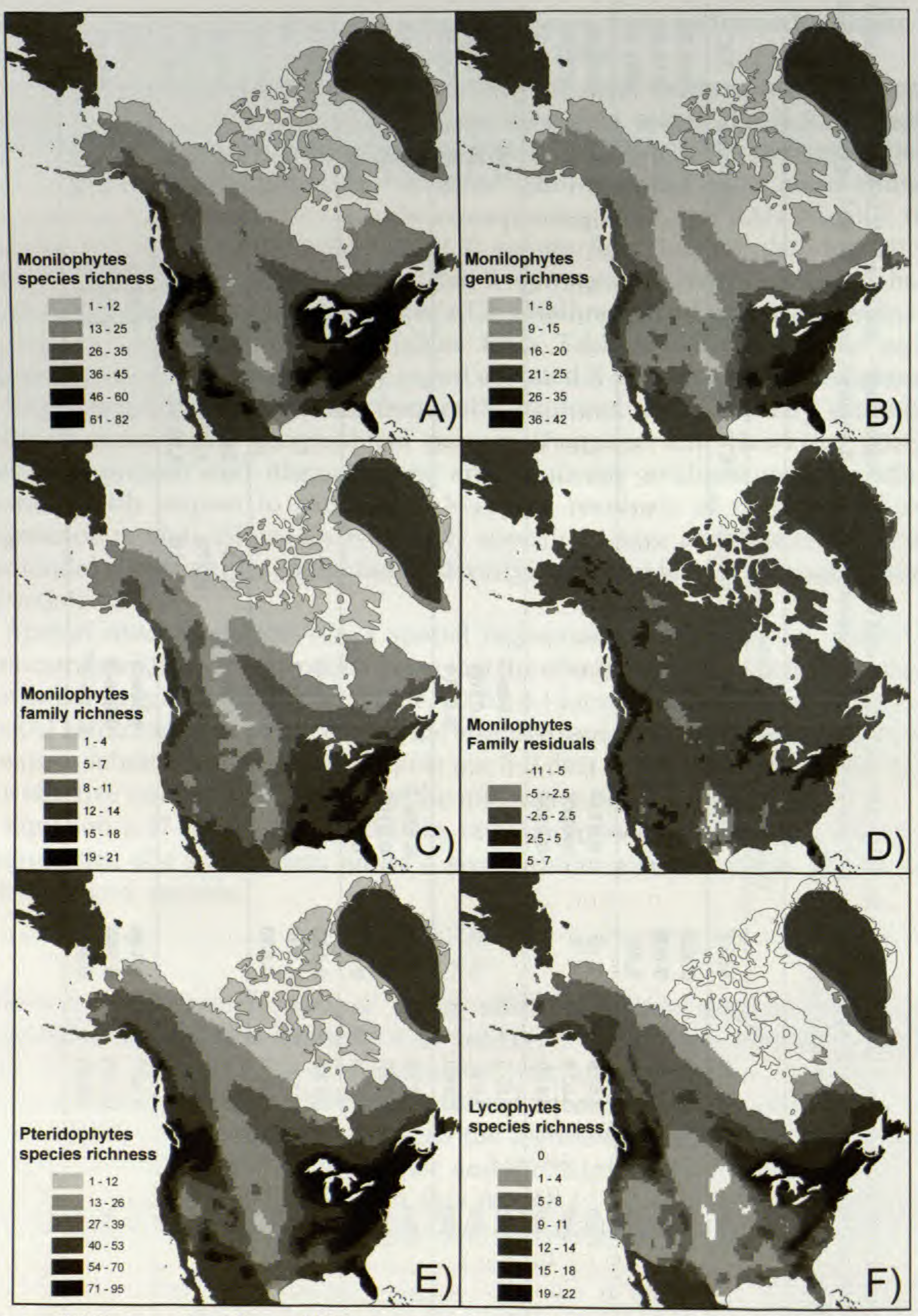


FIG. 1. (A) Fern species richness. (B) Fern genus richness. (C) Fern family richness. Richness values are tabulated by counting numbers of species, genera, or families in 2500 km^2 grid squares. (D) Map of residuals of the regression of fern family richness on mean annual temperature (MAT), annual rainfall (RAN), and precipitation seasonality (Bio15). Projections are Lambert Conformal Conic. (E) Pteridophyte (ferns and lycophytes) species richness. (F) Lycophyte species richness. Color version of maps presented in this paper are available at: http://www.marcbogonovich.org/maps/ferns_lycophytes.html.

Results

Species richness.—A coarse latitudinal pattern in fern species richness is observed with generally higher richness found at lower latitudes (Fig. 1A). Species richness centers occur in the southern half of the continent whereas species poor areas are found in the northern third. Species richness does not reach its peak in a single latitudinal band, nor is the pattern unimodal. Instead there are four richness peaks each with between 60–82 species (*per grid square*). These include two mid-latitude centers, one surrounding Washington state (herein Northwest), which reaches 62 species per square and another surrounding the Appalachians and the Great Lakes region (herein Northeast), which reaches 75 species per square. Two richness centers are identifiable farther south, one centered around Arizona and New Mexico (herein Southwest) reaching 70 species per square and a second, the highest richness peak, in Florida with 82 per square. The Northeastern richness center is the largest in geographical area, stretching from Nova Scotia and New England to the Great Lakes and extending south along the Appalachians. Richness poor regions include the Great Plains, the Canadian Arctic, and a richness trough between Florida and the northeast.

Lycophytes, like ferns, have species richness peaks in the U.S. northeast and northwest, though lycophyte richness and lycophyte richness peaks are comparably more northern, and the Great Plains is a species poor region for both groups. Unlike ferns, lycophytes lack species richness centers in Florida and the Southwest (Fig. 1F). The pattern of pteridophyte species richness is dominated by ferns, the larger constituent taxon (Fig. 1E).

Fern richness at higher taxonomic levels.—There are large differences in patterns of fern richness at the three taxonomic levels (Figs. 1A–C). Richness patterns observed at genus and family levels more closely approach a unimodal pattern than does species richness. The southwestern richness peak observed for species does not exist at the family level, and is much reduced at the genus level. Similarly, the rise in Northwestern species richness relative to surrounding areas is not as large at the family level. The Northeastern species richness center, observed at the species level, shifts south and is less pronounced at the family level and nearly merges with the Floridian richness peak. At the family level there is essentially a single richness center in the eastern United States, reaching its maximum in Florida, the subtropical region of the study area.

Richness-climate relationships.—Regressions between climate variables and fern species, genus, and family richness reveal interesting patterns (Table 2). The strongest relationships found are with fern family richness (with AET, $R^2 = 0.678$; MAT, $R^2 = 0.676$). The same climate variables explain substantially less variation in *species* richness (AET, $R^2 = 0.449$; MAT, $R^2 = 0.539$). Climate relationships are consistently stronger for fern family richness than genus richness. Most of our discussion below on richness-climate models concerns fern family richness.

As expected, multiple regressions incorporating two variables explain more variation in richness than single variable regressions. MAT and AET explain

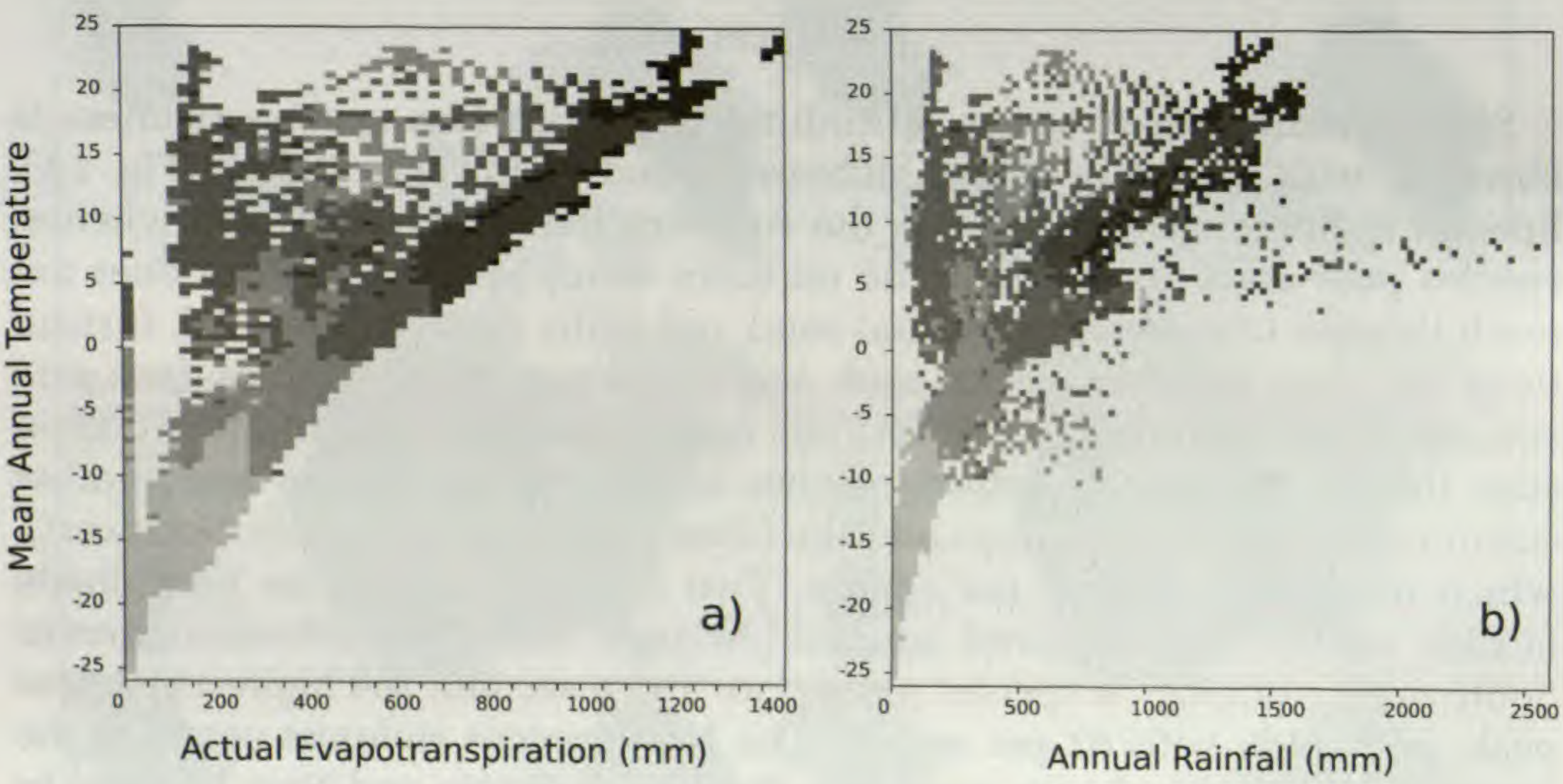


FIG. 2. (a) Binned scatter plot of family richness in North America with actual evapotranspiration (AET) on the x-axis and mean annual temperature is on the y-axis (MAT). Family richness is represented by shade; the shade scale is identical to Fig. 1C. In generating this plot the points are the 2500 km^2 grid squares. These points are binned into x-y dataspace cells, the average value of points with a particular combination of climate values (represented by bins) determines the z-value, or family richness, represented in the graph with shade. More than one location on the continent may have similar climate values and so the average family richness of these locations will be represented in this figure. (b) Right panel. Otherwise similar to Fig. 2a, except that annual rainfall (RAN) is represented on the x-axis rather than AET.

77.6% and MAT and RAN explain 78.1% of the variation in fern family richness (Table 2; Fig. 2), roughly 10% more than any single variable. Warmer and wetter regions generally contain more fern families (Fig. 2a–b). Warmer regions that are also wet have slightly more families than regions that are warm but not as wet. Additionally, dry regions that are very cold have fewer families than similarly dry regions that are warmer.

These simple multiple regressions incorporating one temperature variable and one liquid water variable slightly out-performed the IGM I ($R^2 = 0.781$ vs. 0.711 with family richness as the response variable). A best subsets regression on the variables included in Table 1, confirmed that the best two-variable model was simply MAT and RAN. The best three variable model included MAT, RAN, and precipitation seasonality (Bio15, Table 1), and explained 80.7% of the variation in fern family richness (Table 2). Again, as found for univariate regressions, the multiple regressions consistently explained more variation in family richness than species richness.

A map of the residuals of the regression of fern family richness as a function of MAT, RAN, and Bio15 (precipitation seasonality) illustrates regions where this regression model over-predicts and under-predicts family richness (Fig. 1D, presented are the residuals of the model using all 8,760 squares). There are more families along western North American coastal mountain ranges than predicted by this regression and fewer fern families in the mid-longitude Great Plains

region of North America. There is some correspondence between positive residuals in this relationship and identified species richness peaks and negative residuals and species richness troughs (Fig. 1A and 1D).

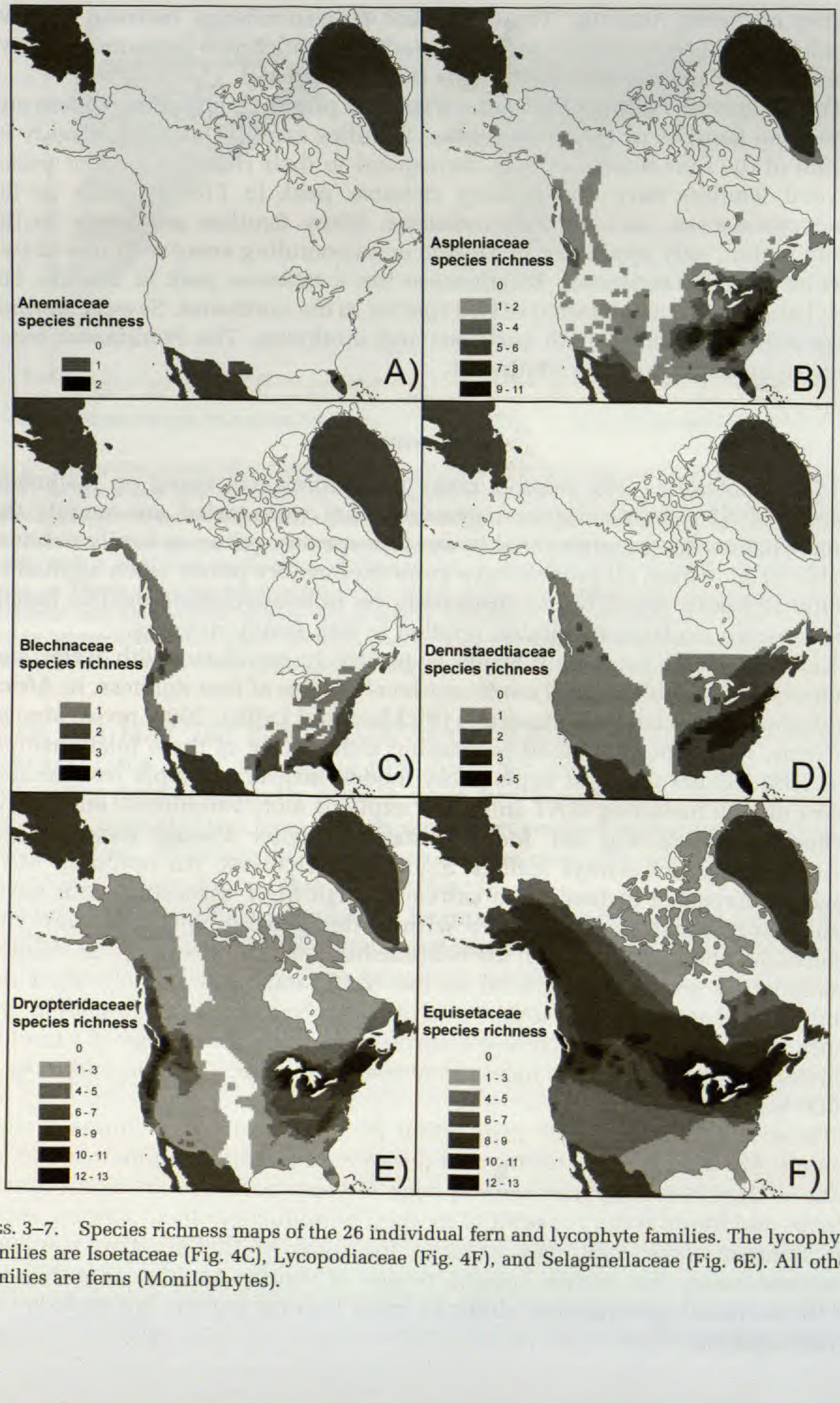
Family species richness patterns.—Figs. 3–7 present maps of the 26 fern and lycophyte families of the study region. Families can be classified roughly by which of the four richness peaks correspond to their richness peak or peaks. Several families have one primary richness peak in Florida, such as the Thelypteridaceae, and the Polypodiaceae. Many families are barely in the study region, only appearing in Florida or surrounding areas with one or two species (e.g., Tectariaceae). Blechnaceae has a richness peak in Florida, but also has a small concentration of two species in the northwest. Several families have richness peaks in both northeast and northwest. The Pteridaceae has a large richness peak in the southwest.

Discussion

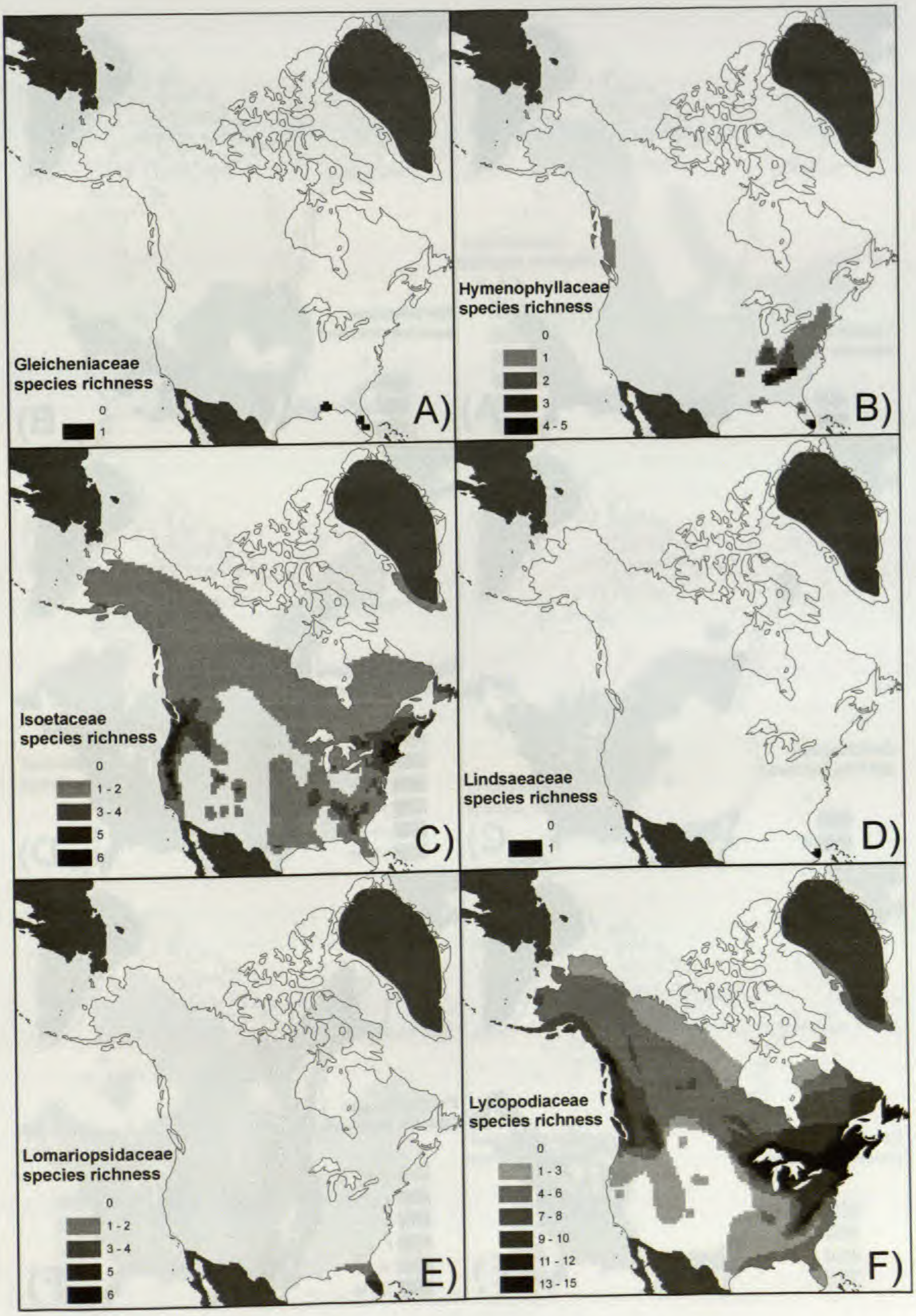
Climate models.—The relative ranks in performance, based on R-squared values, of all richness-climate regressions that were tested are roughly the same whether the response variable was fern species, genus, or family richness (Table 2). However, all models have more explanatory power when applied to family richness. Much of our discussion on richness-climate models herein will concern explanatory models applied to fern *family* richness.

Richness at all taxonomic levels is positively correlated with water and temperature variables. Other continent-level studies of fern richness, in Africa (Aldasoro *et al.*, 2004) and Australia (Bickford and Laffan, 2006) reveal similar patterns, indicating the broad geographic universality of these relationships. The IGM I is not the most explanatory model. Instead, a simple two-variable linear model, including MAT and RAN, explains more variation in fern family richness (Table 2, Fig. 2b). More rainfall or higher average temperatures, however, may not always lead to higher fern richness. An optimum MAT might be expected instead, with extremely high MAT associated with lower fern family richness. However, within the geographical confines and conditions of the study region, the relationship appears linear (Fig. 2). Adding precipitation seasonality (Bio15) to the MAT and RAN model raised the model's explanatory power to 80.7%. This is the best performing model in this study with the model explaining a surprisingly high percentage of variation considering that it does not incorporate spatial climatic variation within each 2,500 km^2 grid square.

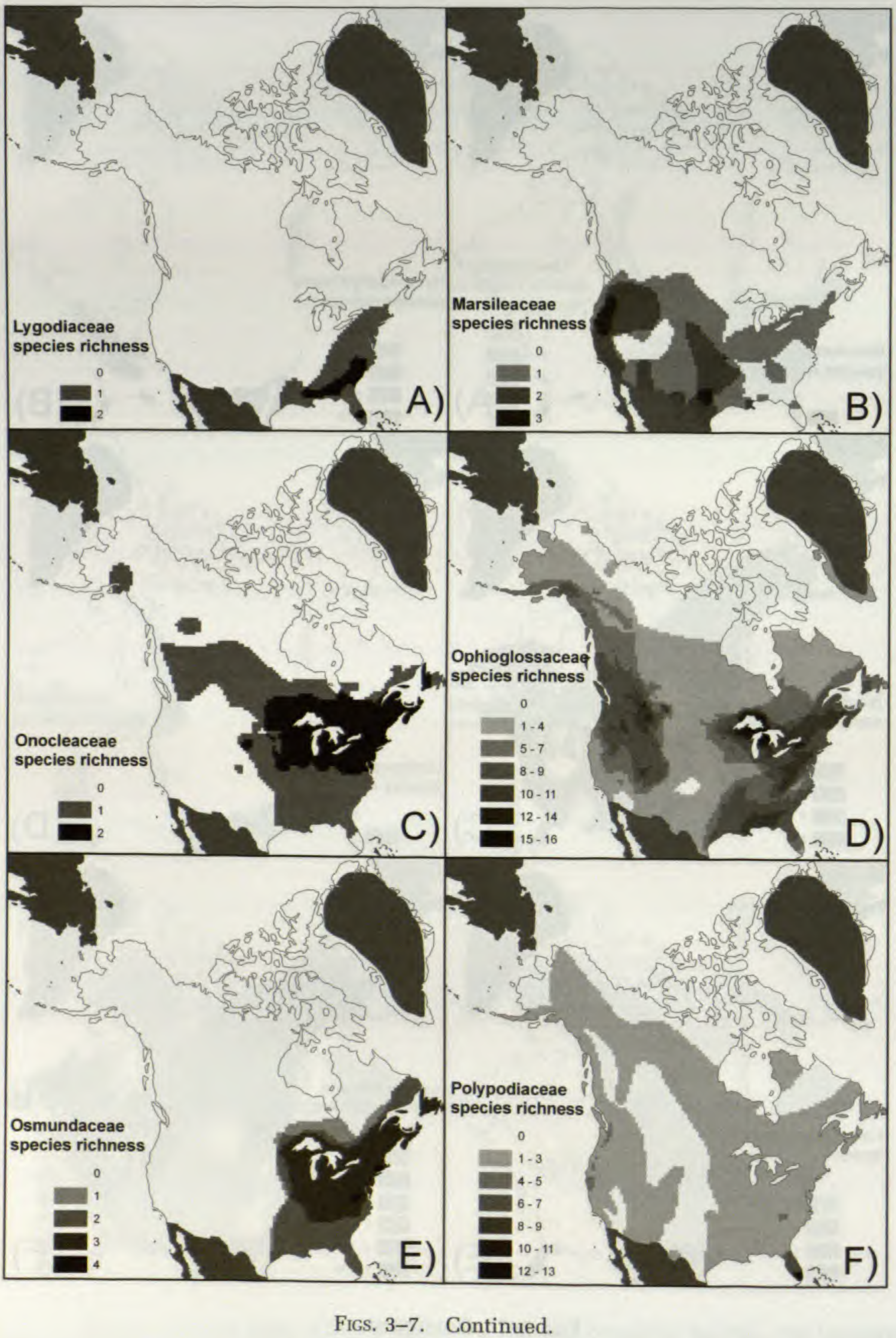
There is further room for exploration of alternative fern richness-climate models. For example, the timing and duration of precipitation may matter for ferns as they lack deep root systems such as those possessed by many woody plants, and liquid water is essential for their reproduction. Incorporating terms for summer snow melt may account for some of the negative residuals observed along the wetter coastal ranges of North America (Fig. 1D). In addition, broad patterns may differ as more tropical regions are included in future studies.



FIGS. 3–7. Species richness maps of the 26 individual fern and lycophyte families. The lycophyte families are Isoetaceae (Fig. 4C), Lycopodiaceae (Fig. 4F), and Selaginellaceae (Fig. 6E). All other families are ferns (Monilophytes).



FIGS. 3–7. Continued.



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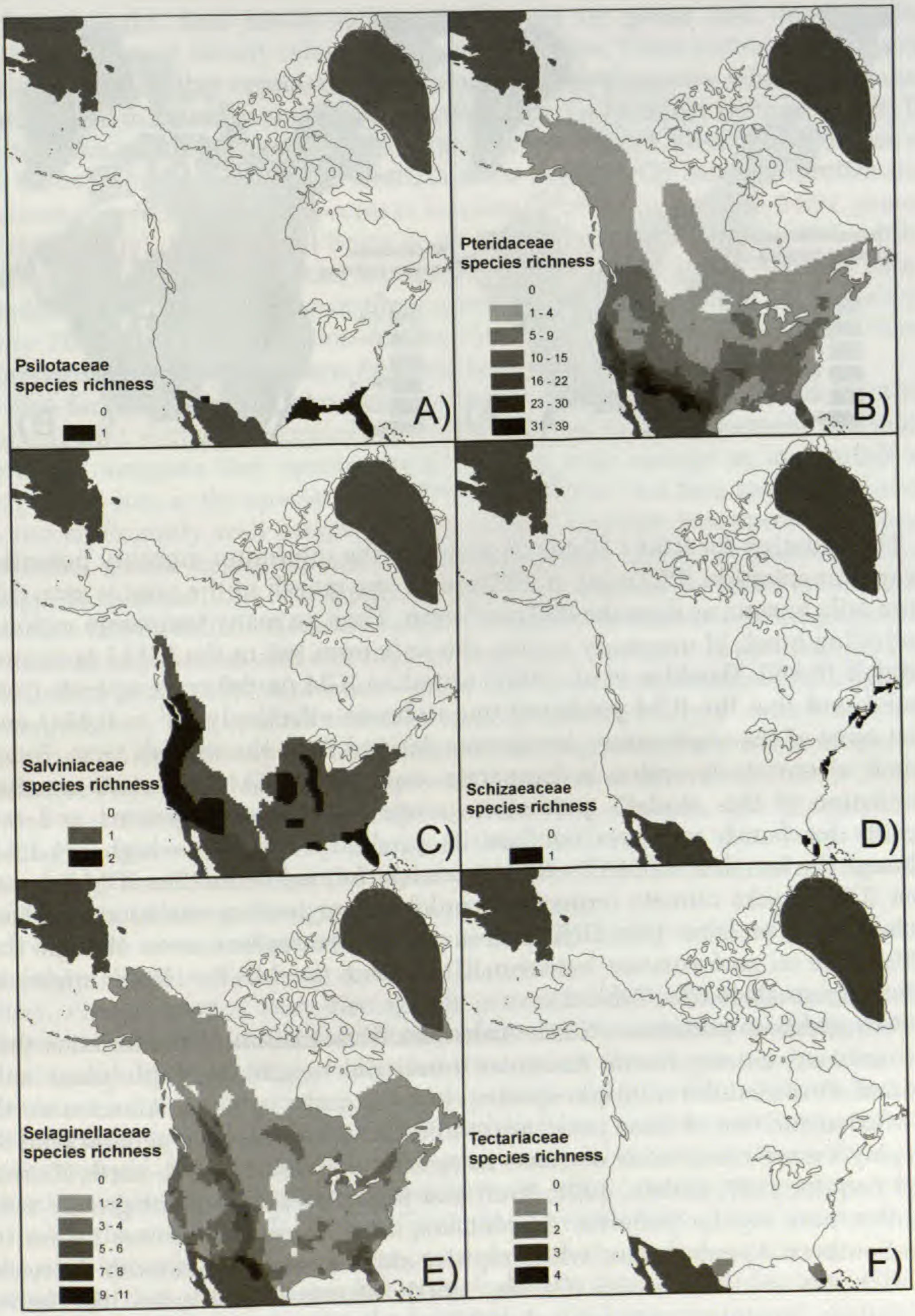

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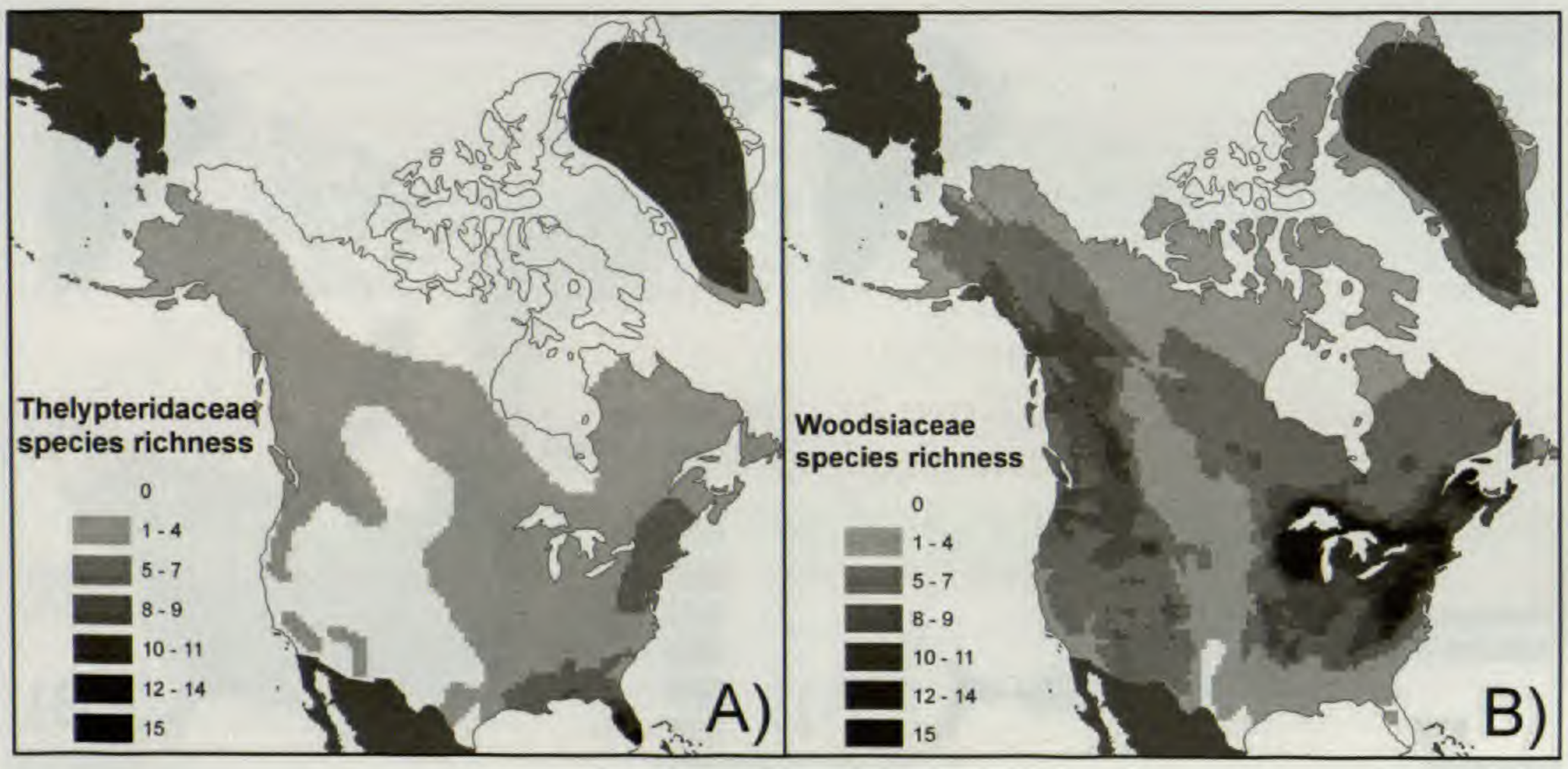

FIGS. 3–7. Continued.

The equation for IGM I (Table 2) includes the minimum monthly potential evapotranspiration (PETmin). If PETmin of one month of the year is zero this term falls to zero, as does the $PETmin^2$ term. Thus, in many temperate regions, including much of our study region, the only term left in the IGM I is annual rainfall (RAN). Hawkins *et al.* (2007) tested an IGM model on temperate trees and found that the IGM predicted tree richness effectively ($R^2 = 0.651$) and that most of the explanatory power was derived from the rainfall term. Since much of our study region is temperate, our test of IGM represents another validation of this model's predictive power in temperate systems and our results for family richness confirm its predictive power is high (71.1%). Though the fact that the MAT + RAN model performed better than IGM I shows that IGM I lacks climate terms that could further resolve variation in plant richness, or at least fern richness, in temperate regions even though the difference in performance between IGM I and the MAT + RAN model is relatively small (9.6%, Table 2).

Fern richness patterns.—North American ferns exhibit characteristics that are unusual among North American terrestrial organisms, both plant and animal. Ferns exhibit multiple species richness peaks in North America north of Mexico and two of these peaks are at mid-latitudes. Plants in general tend to display a more continuous decrease in species richness south to north (Currie and Paquin, 1987; Currie, 1991; Kreft and Jetz, 2007). Non-plant groups also exhibit more regular patterns. Amphibians have a species richness center in the Southern Appalachians, while reptiles show a relatively smooth decrease in richness south to north (Currie, 1991). Mammals are more like ferns, exhibiting irregular geographical richness patterns (Simpson, 1964). The multi-modality of fern species richness peaks may contribute to the low climate-species richness relationship relative to the family level relationship. Fern family richness patterns resemble the patterns seen in other groups more than fern species richness.

Surprisingly, fern *family* richness, followed by genus and then species richness, is most closely related to climate variables. These patterns differ from those found in other organisms, or in ferns from other regions, where R-squared values tend to decrease or stay the same from species to family richness levels. In a stepwise multiple regression including several climate variables, Aldasoro *et al.* (2004) found a decay in the strength of the relationship between climate and African fern richness from species to families (R^2 = 0.676, species; 0.654, genus; 0.584, family). Similarly, O'Brien *et al.* (1998) explained roughly 79% of the variation in Southern African woody plant species and genus richness using models including either two or three water and energy variables; but explained only 70–75% of the variation for families, depending on the model. These comparisons make the pattern reported here more puzzling.

The fact that family richness patterns are similar to those found in other regions and with other organisms, while species richness patterns are more unusual, suggests that species level patterns may emerge as an artifact of imperfect data at the species level. It is conceivable that fern *species* richness is not sufficiently well sampled to adequately describe fern species richness patterns, which might explain the weaker species richness-climate relationships. In contrast, the fern family richness pattern may be better understood because family distributions are relatively rapidly determined in the process of botanical exploration and can thus be used as a surrogate of species richness (Balmford *et al.*, 1996). However, studies of botanical exploration challenge the interpretation that species richness may be insufficiently sampled in North America north of Mexico. Pteridologists can determine the *pattern* of species richness even before full sampling, even in regions as diverse as Bolivia (Soria-Auza and Kessler, 2008). The region of the U.S., Canada, and Greenland is probably more completely sampled than Bolivia, and has fewer ferns, providing confidence that the observed pattern of North American species richness is an adequate signal of the true pattern.

There are several other reasons why genus and family richness may be more strongly related to climate variables than is species richness in this study. First, characteristics that distinguish genera or families may mediate the impact climate has on the distribution of species more than traits that typically distinguish species. Many traits that distinguish fern species seem trivial as environmental adaptations (e.g., sori position on pinnae), while traits that distinguish genera include frond architecture and trophic type of the gametophyte. Second, families and genera may be more evolutionarily stable than species or, at least, less sensitive to short time-scale perturbations. Finally, the mid-latitude richness peaks might result from glacial processes, as the mid-latitude richness centers coincide with the glacial maximum. Glacial processes that promote speciation include recolonizations, founder effects, isolation, hybridization and polyploidization. Cold temperatures are known to foster polyploidization in plants (Otto and Whitton, 2000) and polyploidization is common among ferns (Wood *et al.*, 2009). High fern richness could have been generated by these processes in the last glaciation or by accumulation over repeated cycles of glaciation.

Broader scales may put these patterns in perspective. Mexico has over 1,000 fern species (Mickel and Smith, 2004), more than twice as many as in the much larger, more northern study region. On a global scale, fern species richness patterns may be less unusual and species richness-climate relationships stronger, as temperate richness centers are swamped out by much larger richness centers closer to the Equator. Fern richness-climate regression models should be tested in future studies that incorporate larger areas and lower latitudes.

Further observations.—The proportion of the continental fern flora found in highly diverse regions differs among taxonomic levels. The highest fern species richness is found in regions around Florida followed by the northeast. They contain 82 and 75 species, respectively, or ~20% of the continental pool of 387 species. The percentages increase with taxonomic level. The highest concentrations of genera and families occur in Florida, with 42 genera, or 62.7% of the continental pool of 67 genera, and 21 families, or 91.3% of the pool of 23 families. Together with the climate relationships, this suggests that most fern families are tropical or subtropical in origin with differential expansion (of families) into temperate regions. This can be thought of as a climatic filtering of families toward regions of lower rainfall and temperature. This pattern is consistent with the out of the tropics hypothesis (OTT) articulated by Jablonski (1993) and Jablonski *et al.*, (2006) to explain the evolutionary construction of the latitudinal diversity gradient. However, this pattern is also consistent with the tropical conservatism hypothesis (TCH; Wiens and Donoghue, 2004).

The species richness patterns of individual fern and lycophyte families may provide clues about overall fern and lycophyte richness patterns. The Southwest species richness center (Fig. 1A) is largely composed of species from one family, the Pteridaceae (42 out of 70; Fig. 6B); many of which are triploids. This pattern has been recognized before (Tryon, 1969). On the other hand the Northeast richness center appears to be the result of high numbers of species from *several* families including the Dryopteridaceae, Osmundaceae, and Ophioglossaceae among others. Together these observations suggest that multiple explanations may be required to explain fern species richness patterns, but also suggests that the northeast and northwest richness peaks are a general phenomenon and not the result of one or two aberrant fern families. Interestingly, two lycophyte families, which are only distantly related to ferns, have richness peaks corresponding to the northeast and northwest fern richness peaks. Other than the Pteridaceae, only the lycophyte family Selaginellaceae (Fig. 6E) reaches its peak richness in the southwest.

Other groups including mammals and other plants (trees) have lower richness in Florida than in surrounding areas (Currie and Paquin, 1987; Simpson, 1964). This pattern has been hypothesized to result from the so-called peninsular effect, related to island biogeography theory (Taylor and Regal, 1978; but see Jenkins and Rhine, 2008). Peninsulas share spatial properties with islands, namely separation and distance from continental species pools. Therefore, like islands, peninsulas are expected to have fewer

species than is typical for the environment due to limitations on dispersal of appropriate lineages into the peninsular regions. In contrast, ferns actually experience a *high richness peak* in Florida. Few Florida fern species are endemic and most also exist in the West Indies or South America, with fewer affinities to the Mexican fern flora. One explanation for the pronounced fern richness center in Florida may involve ferns' long-range dispersal capacity (Spurr, 1941). Other geographical patterns of ferns are consistent with this hypothesis. The vascular plant floras of isolated oceanic islands tend to have higher percentages of pteridophytes than the mainland (Kreft *et al.*, 2010; Tryon 1970). The observed high species richness of ferns and low species richness of other groups in Florida is consistent with the interpretation that ferns are less limited by barriers to dispersal than other groups.

Conclusion.—North American ferns have unusual mid-latitude species richness peaks, while family richness patterns are more comparable to those reported for species in other organisms. The fern species richness peaks occur in diverse climates, with different composition and numbers of higher taxa, suggesting that there may be more than one explanation required to understand fern species richness patterns. In contrast, fern family richness is strongly correlated with a small number of climate variables, suggesting a parsimonious explanation is sufficient to explain family richness patterns.

The best regression model included three variables – mean annual temperature, annual rainfall, and precipitation seasonality – and explained 80.7% of the variation in fern family richness. The result that fern richness, particularly at the family level, is strongly related to water and energy variables supports the most general prediction of the productivity-diversity hypothesis. More work is needed to explore and evaluate alternative fern richness-climate models.

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Chemical Composition of Essential Oils from Two Fern Species of *Anemia*

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ABSTRACT.—Although several species of ferns possess a characteristic odor, only a few published studies have described their volatile constituents. The aim of the present study was to evaluate the chemical composition of essential oils from two chemically unexplored fern species, *Anemia hirsuta* and *Anemia raddiana*. These species were collected during the day in ravine banks of Rio de Janeiro state, Brazil. Fresh leaves of the ferns were individually turbolized with distilled water and submitted to hydrodistillation in a Clevenger-type apparatus for three hours. The essential oils obtained were subjected to gas chromatography/mass spectrometry for identification. Nine compounds were identified in *Anemia raddiana* essential oil, accounting for 81.3% of the total oil, where the sesquiterpene β-selinene (46.8%) was the major compound. Thirteen compounds were identified in *Anemia hirsuta* (92.3% of the total oil) and the sesquiterpene β-caryophyllene was the major compound (48.7%). No similarity was observed between the chemical profile of essential oils obtained from *A. hirsuta* and *A. raddiana*. Likewise, extremely low similarities (≤6.2%) were observed between these species and three previously published profiles of *Anemia tomentosa* var. *anthriscifolia*.

KEY WORDS.—Anemiaceae, essential oil, monoterpenes, sesquiterpenes, fern

Anemia is the only genus in the family Anemiaceae, a member of the order Schizaeales (schizaeoid ferns). The Schizaeales is characterized by fertile-sterile leaf blade differentiation, absence of well-defined sori, and sporangia possessing a transverse, subapical, continuous annulus (Smith *et al.*, 2006; Rothfels *et al.*, 2012). The genus *Anemia* contains about 100–120 species, mainly distributed within the tropical and subtropical regions (Smith *et al.*, 2006; Skog *et al.*, 2002). In Brazil, there are 70 species and seven varieties of *Anemia* (Barros *et al.*, 2013) and many endemic species occur in the southeastern and central part of the country, which is known as the center of this genus diversity (Mickel, 1962; Tryon and Tryon, 1982; Moran and Mickel, 1995). *Anemia* is easily identified by the sporangia on a basal pair of skeletonized, highly modified, erect pinnae (Smith *et al.*, 2006). Skog *et al.* (2002) concluded that species of the genus *Anemia* fall into two well-supported subgenera, *Anemiorrhiza* and *Anemia* (including subg. *Coptophyllum*). Many *Anemia* species have aromatic fronds (Page, 2002; Juliani *et al.*, 2004; Santos and Sylvestre, 2006) that produce volatile substances by glandular hairs (Ribeiro *et al.*, 2007).

Essential oils are volatile substances responsible for the scent, odor or smell found in many plants, and are mainly comprised of terpenes. However, other classes of chemical substances may also be present, such as phenylpropanoids and hydrocarbons (Harborne 1984). Although several species of ferns possess a quite typical smell (Page, 2002), only a few works regarding chemical characterization of their volatile constituents have been reported in the literature (Briggs and Sutherland, 1947; Soeder, 1985; Cheng and Mao, 2005; Miyazawa *et al.*, 2007), with special efforts carried out to study the species *Anemia tomentosa* (Sav.) Sw. var. *anthriscifolia* (Schrad.) Mickel (Juliani *et al.*, 2004; Santos *et al.*, 2006, 2008, 2010; Pinto *et al.*, 2007; 2009a; 2009b; Joseph-Nathan *et al.*, 2010).

The aim of the present study was to analyze the chemical composition of essential oils from two fern species, *Anemia hirsuta* and *Anemia raddiana*. *Anemia hirsuta* (L.) Sw. and *Anemia raddiana* Link. (Fig. 1) occur in ravine banks at the forest edge, in open habitats and also on rocky outcrops. Both species belong to the subgenus *Anemia*. *Anemia hirsuta* has a once-pinnate foliar blade, fertile pinnae approximate to the sterile, free veins, deeply incised pinnae, hirsute, brown rhizome hairs and a Neotropical distribution (Mickel, 1982). *Anemia raddiana* has a bipinnate foliar blade, fertile pinnae that are remote from sterile pinnae, red rhizome hairs and an endemic geographical distribution in Southeastern and Southern Brazil (Mickel, 1962; Schwartsburd and Labiak, 2007). To our knowledge, this is the first phytochemical characterization of essential oils from these two species of ferns.

Materials and Methods

Plant material.—Leaves of *Anemia hirsuta* were collected during the day (March, 2009) in ravine banks at "Serra das Emerências" in the municipality of Armação de Búzios, Rio de Janeiro state, Brazil (22°47′53.2″S,–41°56′ 0.5″W),

FIG. 1. *Anemia raddiana* Link. (A) and *Anemia hirsuta* (L.) Sw. (B).

and a voucher sample was deposited under the register number RFFP – 13.790. Leaves of *Anemia raddiana* were collected (February, 2010) in ravine banks at "Instituto Zoobotânico de Morro Azul", municipality of Engenheiro Paulo de Frontin, Rio de Janeiro state, Brazil (22°29′42.28″S–43°34′2.37″W) and a voucher sample was deposited under the register number RFFP – 13.791. The voucher samples were deposited at the herbarium of Faculdade de Formação de Professores, Universidade do Estado do Rio de Janeiro (RFFP).

Essential oils extraction.—Fresh leaves of *A. hirsuta* (0.7 kg) and *A. raddiana* (0.9 kg) were individually turbolized with distilled water and then submitted to hydrodistillation for three hours using a Clevenger-type apparatus. The resulting essential oils extracts were dried over anhydrous sodium sulphate and stored at approximately 4°C until analysis.

Gas chromatography/mass spectrometry analysis.—One microliter of each sample was dissolved in CH_2Cl_2 (1:100 mg/μL) and analyzed by GC/MS (Shimadzu QP5000) using a DB-5MS column (5% phenylmethyl silicone, 30 m × 0.25 mm ID, 0.25 μm film thickness). Gas chromatographic (GC) conditions were as follows: injector temperature 260°C; detector temperature 290°C; carrier gas (helium), flow rate 1 mL/min and split injection with a 1:40 split ratio. Oven temperature was initially held for 2 min at 60°C, and then raised to 290°C at a rate of 3°C/min. Mass spectra were recorded at 70eV with a mass range from *m/z* 35–450 and scan rate of 1 scan/sec. The Retention Indices (RI) were calculated by linear interpolation to the retention times of the eluting peaks with the retention times of a mixture of aliphatic hydrocarbons (C_5–C_{24})

analyzed in the same conditions (Van Den Dool and Kartz, 1963). The compounds identification was performed by comparison of their retention indices and mass spectra with those reported in literature (Adams, 2007). The MS fragmentation pattern of compounds was also checked with NIST mass spectra libraries. Quantitative analysis of the chemical constituents was performed by flame ionization gas chromatography (GC/FID) under the same conditions of GC/MS analysis and percentages obtained by FID peak-area normalization method.

Data analysis.—Sørensen's similarity index was used to compare the essential oil composition between the *Anemia* species (Brower *et al.*, 1997).

Results

Essential oils were obtained from fresh ferns and yielded 0.04% and 0.03% w/w for *Anemia radianna* and *Anemia hirsuta*, respectively. *Anemia radianna* essential oil was analyzed by GC/MS and nine compounds were identified, corresponding to 81.3% of the total oil composition (Table 1). The major compound was β-selinene (46.8%). Thirteen substances were identified by GC/MS analysis of *A. hirsuta* essential oil (Table 1). The substances corresponded to 92.3% of the total oil composition (Table 1). The most abundant constituent was the sesquiterpene β-caryophyllene (48.7%). The remaining substances found in both essential oils are listed in Table 1. No similarity between the essential oils constituents of *A. hirsuta, A. radiana* were detected (Tables 1 & 2).

Discussion

The present study found that the essential oils from *Anemia hirsuta* and *Anemia radianna* are mainly composed of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes.

The sesquiterpene β-caryophyllene, which is the most abundant constituent of the essential oil of *A. hirsuta*, is recognized by its antifeedant and insecticidal activity (Barakat, 2011; Glinwood *et al.*, 2011); it also displays phytotoxic activity (Glinwood *et al.*, 2011) and can accumulate in soil (Asensio *et al.*, 2008). Similarly, β-selinene, the major substance found in essential oil from *A. radianna*, is known to have allelopathic effects (Bewick *et al.*, 1994).

Many terpenoids are known to possess allelopathic properties (Reigosa *et al.*, 2006), mediate direct and indirect plant defenses (Pichersky and Gershenzon, 2002) and improve thermotolerance in plants (Sharkey *et al.*, 2001). Extracts obtained from *A. villosa* and *Anemia tomentosa* var. *anthriscifolia* were able to inhibit germination and development of lettuce seedlings (Moraes *et al.*, 2003). Fern species, among them *Pteridium aquilinum* (L.) Kuhn and *Dicranopteris linearis* (Burm. f.) Underw. produce allelopathic substances in order to form dense populations (Walker and Sharpe, 2010). Several classes of secondary (or special) metabolites can be allelochemical

TABLE 1. Constituents of essential oil from two *Anemia* species and its percentage composition. RI-Retention Indices on DB-5MS column in reference to *n*-alkanes. RT-Retention Time (minutes).

RI	RT (min)	Components	*Anemia raddiana*	*Anemia hirsuta*
930	**4.73**	α-pinene	-	2.2
1385	**21.82**	β-elemene	8.8	-
1414	**23.20**	β-caryophyllene	-	48.7
1450	**24.72**	E-β-farnesene	-	4.6
1458	**25.03**	α-acoradiene	-	2.7
1484	**26.00**	β-selinene	46.8	-
1490	**26.27**	δ-selinene	3.8	-
1503	**26.92**	Sandalore	-	4.7
1500	**26.71**	Unknown[a]	2.1	-
1576	**29.91**	Caryophyllene oxide	-	5.0
1613	**31.24**	Unknown[b]	1.9	-
1653	**32.77**	Unknown[c]	3.3	-
1655	**32.86**	Selin-11-en-4-α-ol	11.5	-
1664	**33.20**	Caryophyllene-14-hydroxy-(Z)	10.4	-
1674	**33.73**	n-tetradecanol	-	1.9
1721	**35.35**	Unknown[d]	11.4	-
1882	**43.99**	Unknown[e]	-	1.6
1886	**44.28**	Palmitic acid	-	4.9
1894	**44.81**	Unknown[f]	-	2.9
2064	**47.17**	Unknown[g]	-	3.2
2082	**47.70**	Abietadiene	-	2.4
2111	**48.67**	Trans phytol	-	15.2
	Monoterpene hydrocarbons		-	2.2
	Oxygenated monoterpenes		-	-
	Monoterpenes: total		-	**2.2**
	Sesquiterpene hydrocarbons		59.4	56.0
	Oxygenated sesquiterpenes		21.9	9.7
	Sesquiterpenes: total		**81.3**	**65.7**
	Other		-	**24.4**
	Total identified		**81.3**	**92.3**

[a] MS *m/z* (relative intensity): 41, 53, 67(100), 79, 93, 107, 119, 133, 147, 161, 189.
[b] MS *m/z* (relative intensity): 41, 43(100), 67, 81, 95, 109, 123, 135, 147, 161, 189, 204.
[c] MS *m/z* (relative intensity): 41(100), 55, 67, 79, 93, 107, 121, 131, 145, 145, 159, 187, 205.
[d] MS *m/z* (relative intensity): 41(100), 55, 67, 79, 93, 105, 119, 133, 145, 159, 173, 187, 205, 220.
[e] MS *m/z* (relative intensity): 41, 55, 67, 81, 95(100), 107, 121, 135, 191.
[f] MS *m/z* (relative intensity): 55, 67, 81, 95(100), 107, 121, 135, 175, 191.
[g] MS *m/z* (relative intensity): 43, 67, 71(100), 95, 107, 121, 133, 147, 161, 175, 189, 257.

agents, including terpenoids and phenolics substances (Harborne, 1999; Ferreira and Aquila, 2000; Reigosa *et al.*, 2002). More studies should be conducted to evaluate the action of the volatile mixture of *Anemia* species as allelopathic agents. Walker *et al.* (2010) emphasized the importance of allelopathy as an important area for research expansion in fern ecology.

Volatile substances released by plants may directly protect them against insect herbivores or do so indirectly by attracting natural enemies of the herbivores (Paré and Tumlinson, 1999; Pichersky and Gershenzon, 2002). Additionally, plant volatiles may function as a form of communication,

TABLE 2. Similarity following Sørensen's index (%) of the essential oil composition between three *Anemia* species and *Anemia tomentosa* var. *anthriscifolia* growing in three different places.

		A. tomentosa (Juliani *et al.*, 2004)		
A. tomentosa (Santos *et al.*, 2006)		0	***A. tomentosa*** (Santos *et al.*, 2006)	
A. tomentosa (Pinto *et al.*, 2007)	**16.0**	0	***A. tomentosa*** (Pinto *et al.*, 2009 a,b)	
A. raddiana (Present study)	6.2	0	4.8	***A. raddiana*** (Present study)
A. hirsuta (Present study)	5.8	0	4.3	0

eliciting changes in undamaged plants neighboring the damaged plants (Pichersky and Gershenzon, 2002). Page (2002) suggested that the prevalence of undamaged fronds and unusual odors in the genus *Anemia* could be reflective of effective chemical defenses against herbivores. Indirect support of this possibility can be found in Gillig *et al.* (2008), who reported the repellent effects of the essential oil from *A. tomentosa* var. *anthriscifolia* against the *Aedes aegypti,* an important mosquito vector of diseases in tropical countries.

Sharkey *et al.* (2001) suggested that isoprene (C_5 terpene) and monoterpenes increases the thermotolerance of the photosynthetic apparatus enabling recovery from brief episodes of temperatures over 40°C. Some aromatic species of *Anemia,* such as *A. hirsuta* and *A. raddiana,* occur in habitats with high light intensity and temperature and edaphic dryness like rock outcrops (inselbergs) (Porembski and Barthlott, 2000; Santos and Sylvestre, 2006). Rock outcrops are centers of diversity for desiccation tolerant vascular plants, among them, many homoiochlorophyllous ferns, including *Anemia* (Meirelles *et al.*, 1997; Porembski and Barthlott, 2000). These fern species have morphological and physiological strategies to tolerate the extreme environmental conditions of the rock outcrops (Porembski and Barthlott, 2000; Ribeiro *et al.*, 2007). Since monoterpenes and other substances present in their essential oils may facilitate tolerance by *Anemia* to these sources of stress, studies that document their essential oil composition and experiments that identify their functions are an interesting approach to future studies in fern species with aromatic leaves growing in these habitats.

No similarity was observed in the essential oil composition of *A. hirsuta* and *A. raddiana.* Remarkably low similarity in essential oil composition was reported in previous studies of *A. tomentosa* var. *anthriscifolia* (Table 2). The largest similarity (16.0%) in this species occurred between two different populations of *A. tomentosa* var. *anthriscifolia* growing in Brazil (Pinto *et al.*, 2009a,b) and Argentina (Juliani *et al.*, 2004). *Anemia tomentosa* var. *anthriscifolia* is a species with a South American geographic distribution. In Brazil, specimens growing on rocks at sea level were characterized by isoafricanol (76%) in Rio de Janeiro state (Santos *et al.*, 2006) and 9-epi-presilfiperfolan-1-ol (30.6%) in Espírito Santo state (Pinto *et al.*, 2009a,b; Joseph-Nathan *et al.*, 2010) as a major secondary compound, whereas those

growing in Argentina (Córdoba province), at 960m above sea level, were characterized by oxygenated sesquiterpenes with α-bisabolol (51.4%) as the major secondary compound (Juliani *et al.*, 2004). In contrast, the major compound of the essential oil in *A. raddiana* and *A. hirsuta* was β-selinene (46.8%) and β-caryophyllene (48.7%), respectively (Table 1). In all studies above, the essential oils were obtained by hydrodistillation from fresh aerial parts in a Clevenger-type apparatus.

Intraspecific variation in the quantitative and qualitative aspects of essential oil production can be influenced by abiotic factors like water and mineral nutrients and biotic factors such as insect damage (Gershenzon, 1983; Paré and Tumlinson, 1999). Hence, the existence of chemotypes is an important aspect of intraspecific essential oils variation (Thompson *et al.*, 2003).

The phylogenetic relationship between species of *Anemia* is an important factor to be considered when assessing interspecific variation in essential oils. Until recently, three subgenera were recognized within the genus *Anemia*, *Coptophyllum* (that included *A. tomentosa* var. *anthriscifolia* and *A. raddiana*), *Anemia* (that included *A. hirsuta*) and *Anemiorrhiza* (Tryon and Tryon, 1982). More recently, Skog *et al.* (2002) concluded that species of the genus *Anemia* fall into only two well-supported subgenera, *Anemiorrhiza* and *Anemia* (including subg. *Coptophyllum*). In accordance with the classification by Mickel (1962), the subgenus *Coptophyllum* had six sections, and *A. tomentosa* var. *anthriscifolia* and *A. raddiana* were classified in the section *Tomentosae*. This same author noted that many phylogenetic problems exist within this section due to their ability to hybridize. To understand the biodiversity is important to evaluate macro and micromolecular characters, mapping and quantifying chemical and biological data (Gottlieb *et al.*, 1996, 1998). In this context, the chemical data of the essential oils may help future ecological and evolutionary analyses in the genus *Anemia*.

To our knowledge, these are the first contributions to the chemical characterization of the essential oils from *Anemia raddiana* and *Anemia hirsuta*. Thus, as part of our ongoing studies of Brazilian fern species, characterization of essential oils may be helpful in the study of the ecology and chemotaxonomy of *Anemia* and other ferns.

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Helical Cell Wall Thickenings in Root Cortical Cells of Polypodiaceae Species from Northwestern Argentina

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ABSTRACT.—The occurrence of helical cell wall thickenings in fern roots is not well investigated and there are few records about it in the literature. To assess the presence of thickenings and their chemical composition, we studied all species of Polypodiaceae, which grow in northwestern Argentina, using light microscopy, scanning electron microscopy, and fluorescence microscopy. Twenty of the twenty-one species studied showed the thickening in the roots. Only in *Melpomene peruviana* are helical cell wall thickenings absent. All thickenings have cellulose as the main compound. The structure of the thickening may be classified as simple, furcate, or anastomosing. All data presented in this paper corroborate the same structure and chemical composition of thickenings previously reported for Aspleniaceae.

KEY WORDS.—Anatomy, Eupolypods I, ferns, epiphytes, cellulose

The presence of a tissue similar to the velamen has been previously recorded in the outer cortical zone of roots for some Polypodiaceae. Geisenhagen (1901, apud Pandé, 1935) was the first author to describe helical thickenings in cell walls of ferns, namely *Niphobolus penangianus* Hook. Also Pandé (1935) reported thickenings in the root cortical parenchymatic cells of *Niphobolus adnacens* (Sw.) Kaulf. (= *Pyrrosia lanceolata* (L.) Farw.), and observed pits in the cell wall between these thickenings. These thickenings appear as strands internally in the cells. Ogura (1972) reported something similar for roots of *Pyrrosia*, but gave no details.

Schneider (1997) studied the root anatomy of the Aspleniaceae and reported spiral wall thickenings in the outer cortex of some species. He mentioned that these thickenings can also be found in epiphytic ferns such as Grammitidaceae, Polypodiaceae, and in some Vittariaceae, but he did not present additional details. He suggested that these cells with helical thickenings function like the cells of the velamen of Orchidaceae.

Recently, Leroux *et al.* (2011) investigated the structure of these root cortical cells for 96 specimens (representing 72 species) of Aspleniaceae and designated them as *helical cell wall thickenings* (henceforth called 'thickenings'). These authors defined them as simple helical thickenings that branch dichotomously, sometimes forming a net-like pattern. They also determined

the chemical composition of the thickenings and found that they are composed of homogalacturonan, cellulose, mannan, and xyloglucan. They found the thickenings in 43 specimens (28 species) of *Asplenium* examined.

Silva *et al.* (2011) have found similar chemical composition for primary cell walls in the leaves of ferns and lycophytes. They suggested that ferns present a third type of cell wall, composed mainly by mannan. In the same paper, details of this chemical cell wall were presented for *Adiantum raddianum* C. Presl. (Pteridaceae).

According to Smith *et al.* (2006), Polypodiaceae is primarily Pantropical, with some species occurring in temperate regions. It contains about 56 genera and 1200 species. Mostly species are epiphytic but some are saxicolous, and rarely are there terrestrial ones. Polypodiaceae sensu Smith *et al.* (2006) encompass the grammitid ferns, which were previously segregated in their own family, the Grammitidaceae (e.g., Ranker *et al.*, 2004; Schneider *et al.*, 2004a).

Investigations carried out over the past ten years on fern classification (e.g., Smith *et al.*, 2006; Christenhusz *et al.*, 2011) and phylogeny and evolution (e.g., Hasebe *et al.*, 1994, 1995; Pryer *et al.*, 1995, 2001, 2004; Schneider *et al.*, 2004b; Schuettpelz and Pryer 2007; Rothfels *et al.*, 2012) based on molecular and morphological data, improved greatly our knowledge about relationships among the major lineages of ferns, but in none of these studies was the presence of the thickenings cited in Polypodiaceae (Eupolypods I) or Aspleniaceae (Eupolypods II).

During the investigation of the sporophyte anatomy of *Pleopeltis minima* (Bory) J. Prado & R.H. Hirai (Prado and Hirai, 2010), the thickenings in the root parenchymatic cortical cells came to our attention, because there were no recent reports of them in the literature for the Polypodiaceae. Thus, we widened our investigations to include all other species of the family that grow in northwestern Argentina.

The present study has two main goals: First, investigate the presence of thickenings in all species of Polypodiaceae from northwestern Argentina; second, determine their forms and chemical composition, as well as discuss their importance in ferns.

Materials and Methods

The specimens studied in this work came from LIL, MCNS, and LP herbaria, and living plants collected by the authors from several localities in northwestern Argentina. Vouchers for all recently collected species were deposited at LIL and MCNS (Table 1). Root fragments of the specimens were sampled for the anatomical and histochemical studies (Table 1, Figs. 1, 3, 4, 5).

Light microscopy.—Anatomical specimens were prepared with dried and fixed material. All material removed from the herbarium specimens was rehydrated in boiling water to which a few drops of detergent had been added, and left at 30° C into oven for 24 hours.

To observe the isolated cells containing the wall thickenings, the root tissues were macerated using a mixture of nitric acid plus water (1:1), at room temperature for 25–30 days. The dissociated material was washed five times with distilled water, and stained with Astra Blue (Biopack) and alcoholic Safranine O (basic red) solution (1%).

To determine the chemical composition of the thickenings in root cortical cells, the material was sectioned by hand longitudinally and transversally, and coloured with different polychromatic dyes: aquatic solution 1% of Cresyl Violet and 1% aquatic solution of Cresyl Blue.

To evaluate the width of the thickenings and their number per cell, 10 measurements were made from each specimen, selecting in all cases the five thickest roots of each individual. Maximum and minimum values as well as average and standard deviation were obtained for each species studied (Table 2, Fig. 2A–B).

To test for the presence of lignin in the thickenings, transverse and longitudinal sections were stained with phloroglucinol (1%) with HCl (25%) (D'Ambrogio de Argüeso, 1986). Also, a differential coloration stain was used: Safranine (1%) and Astra Blue (0.5%) (Bruno *et al.*, 2007).

To evaluate the presence of cellulose as the principal chemical component of the - thickenings in root cortical cells, hand-cut longitudinal sections were clarified in a solution of commercial sodium hypochlorite plus water (1:1). After being clarified, the sections were washed five or six times with distilled water and mounted on slides containing 0.05% weight/volume solution of optical fluorescent brightener 28 calcofluor in a sodium phosphate buffer at pH 8 (Romero and Minter, 1988). This fluorescent dye reacts with cellulose. These slides were observed under an Olympus BX51 light microscope with UV filter to fluorescence and under refrigerated camera Olympus DP72. Photographs of the sections were taken in conditions of fluorescence, light, and darkness. In order to obtain cell measurements the software Olympus Cellsens was used. Basically, the width of the thickenings were measured for each species (Table 2).

To test for the presence of suberin in the thickenings, transverse and longitudinal fresh sections of roots were put in ethanol for a few seconds. Later, they were incubated for 5–10 minutes in fresh Sudan III or IV alcoholic solution (2%); differentiated with ethanol 50% and mounted in glycerine (Ruzin, 1999).

All the semi-permanent slides were mounted with water plus glycerine (1:1).

Other observations and photographs were taken under light microscopy Standard 16 Zeiss and Zeiss Axiostar with a camera Cannon Powershot A620 of 7.2 MP attached, plus a Software Cannon Powershot and Olympus CX41 microscope with a digital camera Mshot (MC30). Measurements were performed with the software Mshot Digital Imaging System, at a magnification of 630 X.

Scanning electron microscopy.—Longitudinal hand-cut sections of roots were fixed with 2% glutaraldehyde in phosphate buffer for 72 hours and dehydrated in a graded ethanol series (50%, 70%, 90%, 100%, for 20 min. in each solution). After fixation and dehydration the samples were critical point dried with carbon dioxide (Denton Vacuum DCP-1). Dried sections were fixed

TABLE 1. Studied taxa, methods, measurements of the thickenings, vouchers, and locality. T=terrestrial, E=epiphyte, R=rupicolous, S=saxicolous; Se=scanning electron microscopy, LM=light microscopy; FM=Fluorescence Microscope, SD= longitudinal or cross sections with specific dyes, D=Dissociation, Ph=Phloroglucinol/HCl test, Su=Sudan III and Sudan IV tests.

Taxon	Habit	Plant size cm	Vouchers	Locality	Methods
Campyloneurum aglaolepis (Alston) de la Sota	E	21–40	Martínez 1442 (MCNS)	Jujuy. Dpto Ledesma, P.N. Calilegua, RP. 83.	LM,SD,Su
			Hernández 1793 (LIL)	Tucumán, Dpto. Chicligasta, La Banderita	LM,SD
			Hernández 1930 (LIL)	Tucumán, Dpto. Tafí Viejo, San Javier	LM,D
			Hernández 2192 (LIL)	Tucumán, Dpto. Chicligasta, La Banderita	LM,SD,Se,Ph
			Hernández 2342 (LIL)	Jujuy, Dpto. Dr. Manuel Belgrano, Yala.	LM,SD,Ph
Campyloneurum lorentzii (Hieron.) Ching.	E/R	43–90	Chambi 233 (MCNS)	Salta, Dpto. Capital, San Lorenzo	LM,SD,Se,Su
			de la Sota 2056 (LIL)	Tucumán, Dpto. Tafi Viejo, San Javier y Taficillo	LM,SD
			Hernández 1173 (LIL)	Tucumán, Tafí del Valle, Río los Sosa	LM,SD,Ph
			Meyer 14038 (LIL)	Tucumán, Dpto. Chicligasta, Pto. Las Pavas y Saladillo	LM,SD,Ph
			Scolnik 21Tu123 (LIL)	Tucumán, Dpto. Tafí Viejo, El Nogalar	LM,SD,D
			Venturi 5891 (LIL)	Tucumán, Dpto Tafí Viejo, Cumbre de Taficillo.	LM,SD
Campyloneurum tucumanense (Hieron.) Ching.	R	53–100	Brown s/n (LIL)	Jujuy, Dpto. Ledesma, P. N. Calilegua.	LM,D
			Castillón 615 (LIL)	Tucumán, Dpto. Famaillá, Qda. de Caspinchango	LM,SD
			Castillón 11647 (LIL)	Tucumán, Dpto. Famaillá, Qda de Caspinchango	LM,SD
			de la Sota 1672 (LIL)	Tucumán, Dpto. Tafí del Valle, Río Los Sosa.	LM,SD,Ph
			Legname 109 (LIL)	Tucumán, Dpto. Tafí del Valle, La Angostura	LM,SD,Ph
			Martínez 236 (MCNS)	Jujuy, Dpto. Ledesma, P.N. Calilegua	LM,SD,Su
Lellingeria tungurahuae (Rosenst.) A.R. Sm. & R.C. Moran	E	10–15	Ganem 167 (LP, MCNS)	Salta, Dpto. Santa Victoria, P.N. Baritú	LM,SD,Se,D, Ph,Su
Melpomene peruviana (Desv.) A.R. Sm. & R.C. Moran	S	3.5–7	Hernández 1619 (LIL)	Jujuy, Dpto. Valle Grande, Santa Ana.	LM,D,Ph
			Martínez 1915 (MCNS)	Salta, Dpto Guachipas, Cuesta del Lajar	LM,SD,Se,Ph,Su
			Ruiz 4 (LIL)	Tucumán, Dpto Tafí del Valle, Valle de la Ciénaga	LM,SD
			Schreiter 3115 (LIL)	Tucumán, Dpto. Tafí del Valle, La Ciénaga.	LM,SD
			Venturi 3016 (LIL)	Tucumán, Dpto. Chicligasta, Estancia Las Pavas	LM,SD
Microgramma mortoniana de la Sota	E/R	10–15	Martínez 651 (MCNS)	Salta, Dpto. Santa Victoria, Los Toldos	LM,SD,Se
			Martínez 1238 (MCNS)	Salta, Dpto. Pampa Grande	LM,SD,Ph,Su
			Martínez 1612 (MCNS)	Salta, Dpto. Guachipas, Cebilar	LM,SD,Ph
			Martínez 1912 (MCNS)	Salta, Dpto. Guachipas	LM,SD

TABLE 1. Continued.

Taxon	Habit	Plant size cm	Vouchers	Locality	Methods
Microgramma squamulosa (Kaulf.) de la Sota	E	9–16	Delgado 140 (LIL)	Tucumán, Dpto. Trancas. Vipos	LM,SD
			Hernández 1877 (LIL)	Tucumán, Dpto. Tafí Viejo, Camino de San Javier a Taficillo	LM,SD
			Hernández 1972 (LIL)	Tucumán, Dpto. Tafí Viejo, San Javier	LM,SD,D,Ph
			Hernández 2055 (LIL)	Tucumán, Dpto. Chicligasta Cochuna	LM,SD,Se,Su
			Hernández 2090 (LIL)	Tucumán, Dpto. Chicligasta. Lara	LM,SD,D,Ph
Pecluma barituensis O. Martínez & de la Sota	E	60–80	Brown 1714 (MCNS)	Jujuy, Dpto. Ledesma, P.N. Calilegua	LM,SD,D
			Ganem 163 (MCNS)	Salta, Dpto. Santa Victoria, P.N. Baritú	LM,SD,D,Ph
			Martínez 1836 (MCNS)	Salta, Dpto. Santa Victoria, P.N. Baritú	LM,SD,Ph
			Saravia 8 (MCNS)	Jujuy, Dpto. Ledesma, P.N. Calilegua	LM,SD,Ph,Su
Pecluma filicula (Kaulf.) M.G. Price	E	5–15	Legname 8532 (LIL)	Salta, Dpto. Santa Victoria, Los Toldos	LM,SD
			Legname 9842 (LIL)	Salta, Dpto. Santa Victoria, Los Toldos	LM,SD,Ph
			Martínez 1561 (MCNS)	Salta, Dpto. Santa Victoria, P.N. Baritú.	LM,SD,Su
			Schiavone 11684 (LIL)	Salta, Dpto. Orán, a orillas del Río Pescado.	LM,SD,D
			Schiavone 11990 (LIL)	Salta, Dpto. Santa Victoria, Los Toldos	LM,SD,Ph
Pecluma oranensis (de la Sota) de la Sota	E	30–78	Hernández 87 (LIL)	Tucumán, Dpto. Trancas, Hualinchay	LM,SD
			Legname 8761 (LIL)	Salta, Dpto. Santa Victoria, Parque Nac. Baritú.	LM,SD,Ph
			Legname 9598 (LIL)	Salta, Dpto. Santa Victoria, Los Toldos	LM,SD,D
			Palací 1107 (MCNS)	Salta, Dpto. Capital, Castellanos a 5 km del puente	LM,SD,Se,Ph,Su
			Schiavone 11987 (LIL)	Salta, Dpto. Santa Victoria, Los Toldos	LM,SD
Pecluma venturii (de la Sota) M.G. Price	E	25–60	Brown 1029 (MCNS)	Salta. Dpto. Anta, Parque Nacional El Rey	LM,SD,Su
			Capurro 106 (LIL)	Tucumán, Dpto. Chicligasta, Alpachiri	LM,SD
			Castillón 11650 (LIL)	Tucumán, Dpto. Famaillá, Qda. Caspinchango	LM,SD,D
			Meyer 15107 (LIL)	Tucumán, Dpto Chicligasta, Alpachiri	LM,SD
			Meyer 1893 (LIL)	Tucumán, Dpto. Chicligasta, entre Ptos. Sta. Rosa y Jaya	LM,SD,Ph
			Venturi 2974 (LIL)	Tucumán, Dpto. Chicligasta, Alpachiri	LM,SD

TABLE 1. Continued.

Taxon	Habit	Plant size cm	Vouchers	Locality	Methods
Phlebodium areolatum (Humb. & Bonpl. ex Willd.) J. Sm.	E/R	30–100	Hernández 517 (LIL)	Tucumán, Dpto. Chicligasta, Cochuna	LM,SD
			Hernández, 519 (LIL)	Tucumán, Dpto. Chicligasta, Cochuna	LM,SD,D,Ph
			Hernández 1392 (LIL)	Tucumán, Dpto. Tafí Viejo, San Javier	LM,SD,Ph
			Hernández 2046 (LIL)	Tucumán, Dpto. Chicligasta, Cochuna	LM,SD,Se,D
			Delgado 115 (LIL)	Tucumán, Dpto. Yerba Buena, Horco Molle	LM,SD,D,Ph,Su
			Delgado 165 (LIL)	Tucumán, Dpto. Famaillá	LM,SD
Pleopeltis bryopoda (Maxon) de la Sota	S	6–23	de la Sota 1771 (LIL)	Tucumán, Dpto. Tafí del Vallle, La Quebradita	LM,SD,Ph
			de la Sota 1776 (LIL	Tucumán, Dpto. Tafí del Valle, La Quebradita.	LM,SD
			Martínez 565 (MCNS)	Salta, Dpto. Chicoana, Valle Encantado	LM,SD,Ph,Su
			Venturii 2048 (LIL)	Tucumán, Dpto. Chicligasta, Estancia Santa Rosa	LM,SD,D
			Venturi 4802 (LIL)	Tucumán, Dpto. Chicligasta, Estancia Santa Rosa	LM,SD
Pleopeltis hirsutissima (Raddi) de la Sota	E	19–35	Ibáñez s/n (LIL)	Tucumán, Dpto. Monteros, El Nogalal	LM,SD,D,FM,Ph
			Peréz 21010 (LIL) *	Misiones, Dpto. Loreto	LM,SD,FM,Ph,Su
			Vanni 841 (LIL) *	Misiones, Dpto. Cainguás, Aristóbulo del Valle	LM,SD,D
			Schwarz 6197 (LIL) *	Misiones, Dpto. San Ignacio, Puerto España	LM,SD
			S/N 10525 (LIL) *	Misiones, Dpto. Salto Iguazú	LM,SD,D,Ph
Pleopeltis macrocarpa (Bory ex Willd.) Kaulf.	E	17–30	Frangella s/n (LIL)	Tucumán, Dpto. Tafí del Valle, La Angostura.	LM,SD
			Hernández 1171 (LIL)	Tucumán, Dpto. Monteros, Río Los Sosa.	LM,SD,D,Se
			Hernández 1115 (LIL)	Tucumán, Dpto. Trancas, Hualinchay	LM,SD,Ph,FM
			Meyer 14003 (LIL)	Tucumán, Dpto. Chicligasta, Estancia Las Pavas	LM,SD
			Martínez 555 (MCNS)	Salta, Dpto. Capital, Qda. de San Lorenzo.	LM,SD,Ph,Su
Pleopeltis minima (Bory) J. Prado & R.Y. Hirai	E/R	4–10	Chambi 263 (MCNS)	Salta, Dpto. La Caldera, Vaqueros	LM,SD,Su
			Hernández 755 (LIL)	Tucumán, Dpto. Burruyacu, A° Cajon. Sa. del Campo	LM,SD,Ph
			Hernández 788 (LIL)	Tucumán, Dpto. Capital, en arbolado urbano	LM,SD,Ph
			Hernández 803 (LIL)	Tucumán, Dpto. Capital, en arbolado urbano	LM,SD,D,Ph,Su
			Hernández 805 (LIL)	Tucumán, Dpto. Capital, en arbolado urbano	LM,SD,D,Su
			Schreiter 3295 (LIL)	Tucumán, Dpto. Trancas, El Cadillal	LM,SD,D
Pleopeltis pinnatifida Gillies ex Hook. & Grev.	E/R	11–23	Hernández 165 (LIL)	Tucumán, Dpto. Tafí del Valle, Infienillo.	LM,SD
			Maruñak 345 (LIL)	Tucumán, Dpto. Tafí del Valle	LM,SD,D
			Rodríguez 912 (LIL)	Tucumán. Dpto. J.B. Alberdi, Escaba. Rio Echeverría	LM,SD,Ph
			Ruiz 9 (LIL)	Tucumán, Dpto Tafí del Valle, Valle de la Ciénaga	LM,SD
			Tolaba 1105 (MCNS)	Salta, Dpto Iruya, Qda. del Río San Isidro.	LM,SD,Ph,Su

TABLE 1. Continued.

Taxon	Habit	Plant size cm	Vouchers	Locality	Methods
Pleopeltis pleopeltidis (Fée) de la Sota	E	10–21	Cassá 224 (MCNS)	Tucumán; Dpto. Monteros, Qda. del Portugués.	LM,SD,D
			Martínez 1415 (MCNS)	Jujuy, Calilegua	LM,SD,D,Ph,Su
			Martínez 1540 (MCNS)	Salta, Dpto Santa Victoria, El Nogalar	LM,SD,Ph
			Martínez 1651 (MCNS)	Jujuy, Dpto Ledesma, P.N. Calilegua.	LM,S,SD
			Martínez 2054 (MCNS)	Jujuy, Dpto. Yala, Potrero de Yala	LM,SD,Ph
Pleopeltis tweediana (Hook.) A.R. Sm.	E	10.5–33.5	Hernández 612 (LIL)	Tucumán, Dpto. Monteros. La Florida	LM,SD
			Hernández 1967 (LIL)	Tucumán, Dpto. Tafí Viejo, San Javier	LM,SD
			Hernández 2191 (LIL)	Tucumán, Dpto. Chicligasta, La Banderita.	LM,SD,Se,D,FM, Ph
			Martínez 2142 (MCNS)	Salta, Dpto. Rosario de la Frontera, cerca Hotel Termas	LM,SD,Se,Ph,Su
			Venturi 6031 (LIL)	Tucumán, Dpto. Tafí Viejo, Cumbre de Taficillo	LM,SD
"Polypodium" chrysolepis (Hook.)**	R	5–13	Borsini 866 (LIL)	Tucumán, Dpto. Chicligasta, Los Pinos	LM,SD, Ph
			Castillón 2248 (LIL)	Tucumán, Dpto. Tafí del Valle, La Ventanita.	LM,SD,Su
			Meyer 17995 (LIL)	Tucumán, Dpto. Chicligasta, entre Saladillo y El Bolsón	LM,SD
			Sleumer 1852 (LIL)	Catamarca, Dpto. Andalgala, selva de los Alisos	LM,SD,D,Ph
			Venturi 3151 (LIL)	Tucumán, Dpto. Chicligasta, Estancia Las Pavas	LM,SD,Ph
Serpocaulon gilliesii (C. Chr.) A.R. Sm.	E	26–60	Hernández 86 (LIL)	Tucumán, Dpto. Trancas, Hualinchay	LM,SD,Ph,Su
			Hernández 196 (LIL)	Tucumán, Dpto. Tafí del Valle, Carapunco	LM,SD,D
			Hernández 2047 (LIL)	Tucumán, Dpto. Chicligasta, La Banderita	LM,SD,Se,D,Ph
			Martínez 1032 (MCNS)	Salta, Dpto. Sta. Victoria, P.N. Baritú.	LM,SD,Se
			Meyer 14870 (LIL)	Tucumán, Dpto. Chicligasta, El Bolsón	LM,SD

* Species from outside northwestern Argentina.

** "*Polypodium*" *chrysolepis* is a binomial adopted in this paper, but according to the literature (Schneider *et al.*, 2004) it is a taxon of uncertain position among the genera of Polypodiaceae.

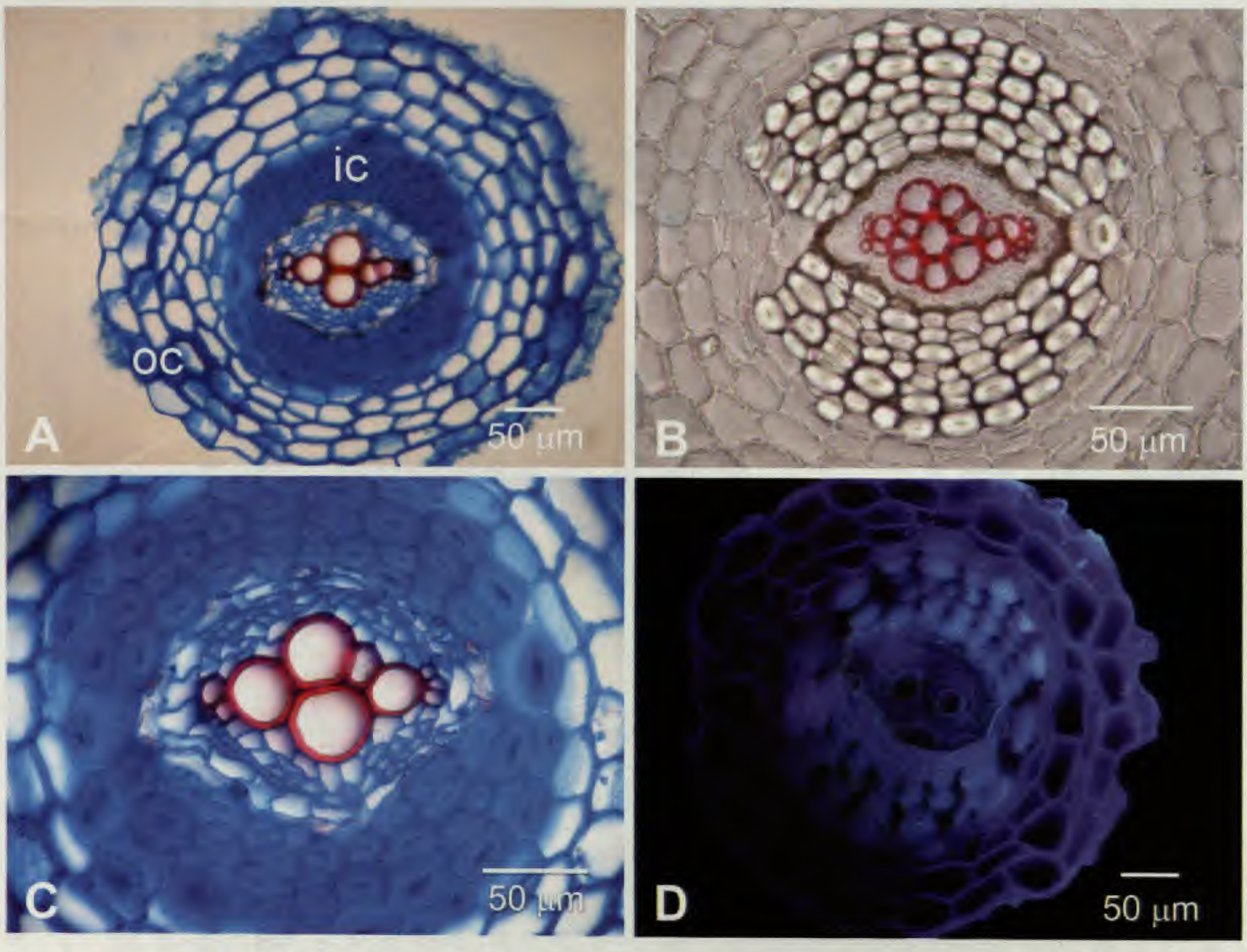

FIG. 1. (A) Transverse section of the root showing: outer cortex (oc), inner cortex (ic). Result for histochemical tests. (B) Negative reaction for Phloroglucinol/HCl to the inner and outer cortex. (C) Differential coloration with Safranin-Astra Blue marked in red lignified cell walls and in blue cellulosic cell walls. (D) Positive reaction of the cellulose with calcofluor, picture took from Fluorescence Microscope. (A, C, D) *Pleopeltis macrocarpa*; (B) *Pleopeltis tweediana*.

on aluminum stubs with double-sided adhesive graphite tape, sputter-coated with gold in a Denton Vacuum Desk IV (20-24 nm thickness) and were observed with a JEOL JSM 6480 LV SEM (Japan), in the Laboratorio de Microscopía Electrónica de Barrido y Microanálisis (LASEM), Universidad Nacional de Salta and JEOL JSM 35 CF and Supra SS VP (Zeiss) in the Laboratorio de Microscopía Electrónica, Universidad Nacional de Tucumán (LAMENOA).

RESULTS

In eight of the genera studied here (Table 1), the root cortex is composed of two clearly differentiated zones: an outer cortex formed by one to eight layers of parenchymatic cells, with primary cell walls that contain the thickenings that surround each cell internally as strands; an inner cortex with three to five layers of sclerenchymatic cells, reduced at the level of the two protoxylematic points where it has one cell, rarely two cells (Fig. 1A). All studied species presented endodermis surrounding the vascular cylinder.

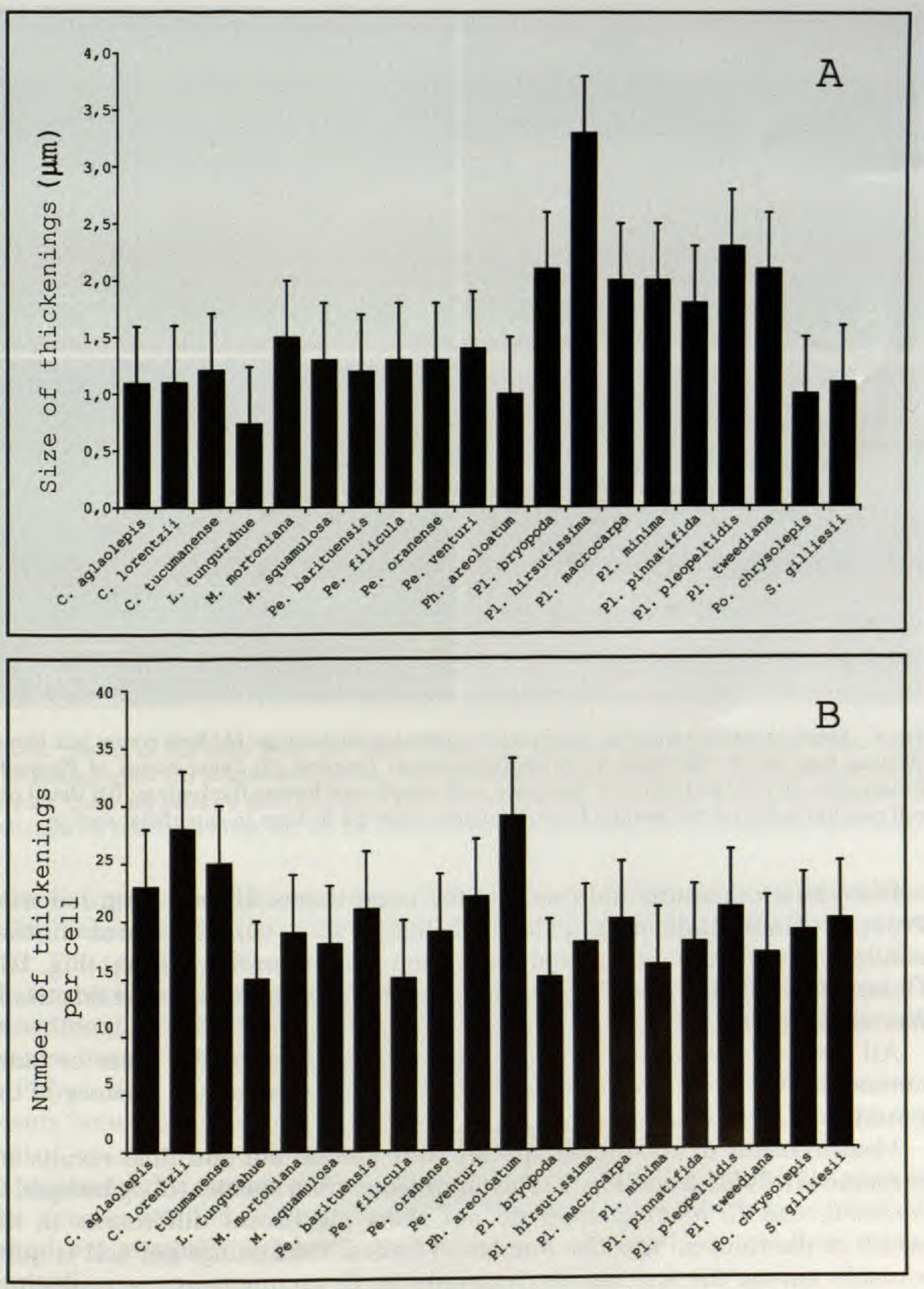

FIG. 2. Mean and Standard deviation (95% confidence intervals) of (A) Thickening's size; (B) Number of thickenings per cell.

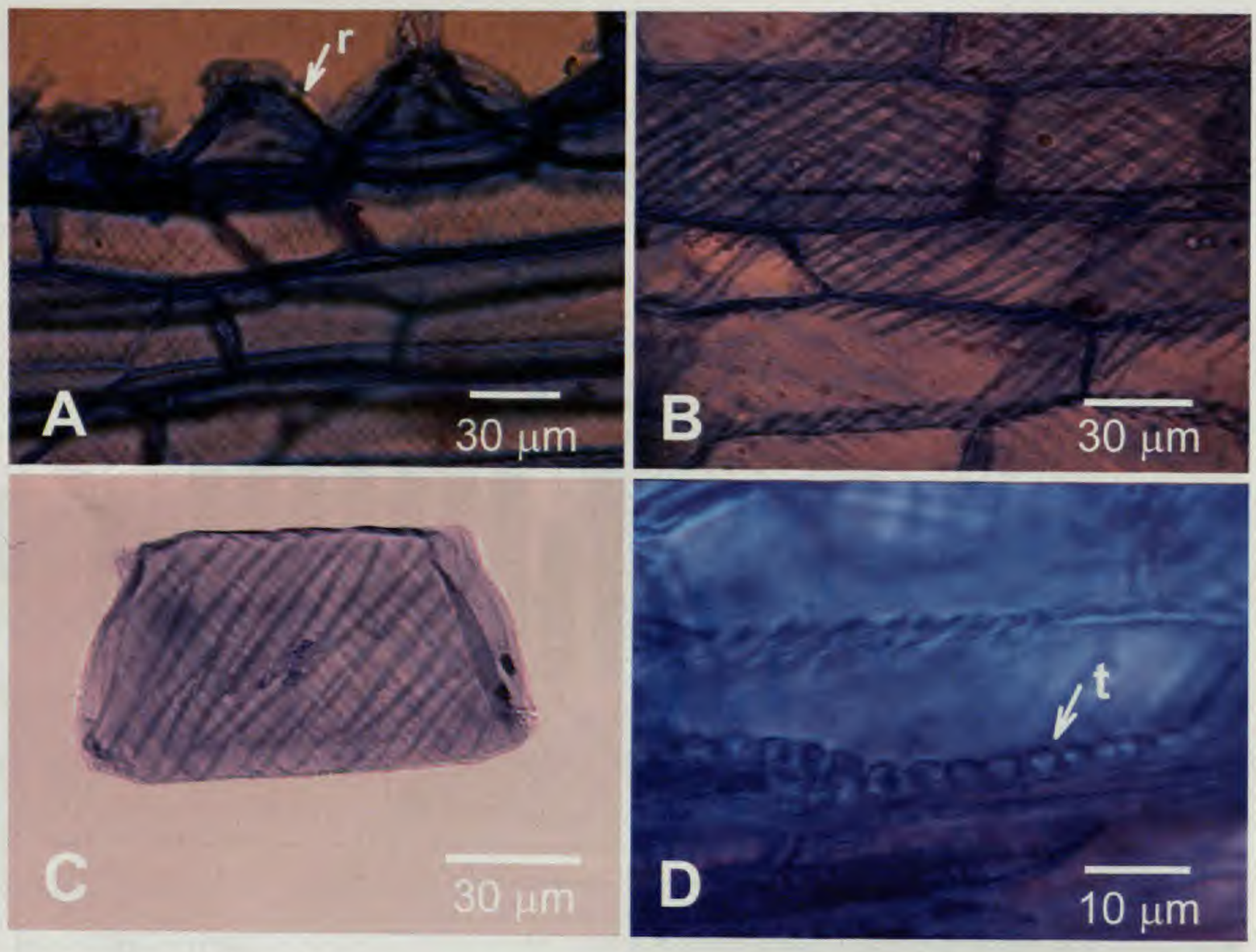

FIG. 3. Detail of the cells from the outer cortex containing thickenings. (A) Root cortex lack lignin, showing base of the root hairs (r) of *Campyloneurum lorentzii*; (B) Outer cortex of *Pleopeltis hirsutissima*; (C) Isolated cell of *P. bryopoda* with simple and furcate thickenings; (D) Detail of a cell containing helical thickenings (t) of *P. minima*, from 2.5 to 3 μm in superficial section.

Histochemical results indicate that the inner cortex did not stain red with Phloroglucinol, indicating a lack of lignin (Fig. 1B). This was further confirmed with the differential coloration Safranine-Astra Blue (Fig. 1C). Observations made under the Fluorescence Microscope showed that this tissue has cellulose (Fig. 1D).

All tests for the presence of suberin were negative for the inner or outer cortex, but the overall result was positive for the endodermis, because of the presence of the Casparian bands.

Measurements of all studied species were carried out and final results are presented in Table 2 and Fig. 2. The *Campyloneurum* species (*C. aglaolepis, C. lorentzii,* and *C. tucumanense*) do not show significant differences in the width of the thickenings. The number of helical thickenings per cell is quite variable among the species. For example in *C. tucumanense*, a rupicolous species, there are 9–45 thickenings per cell, whereas *C. lorentzii* (Fig. 3A), an epiphytic or rupicolous species 17–40 strands. Compared to other species, *Lellingeria tunguraguae* showed the narrowest thickenings (0.5–1.2 μm) and least number of thickenings per cell. The species of *Pecluma* (*P. barituensis*, *P. filicula*, *P. oranensis*, and *P. venturii*), all epiphytes, have similar thickenings,

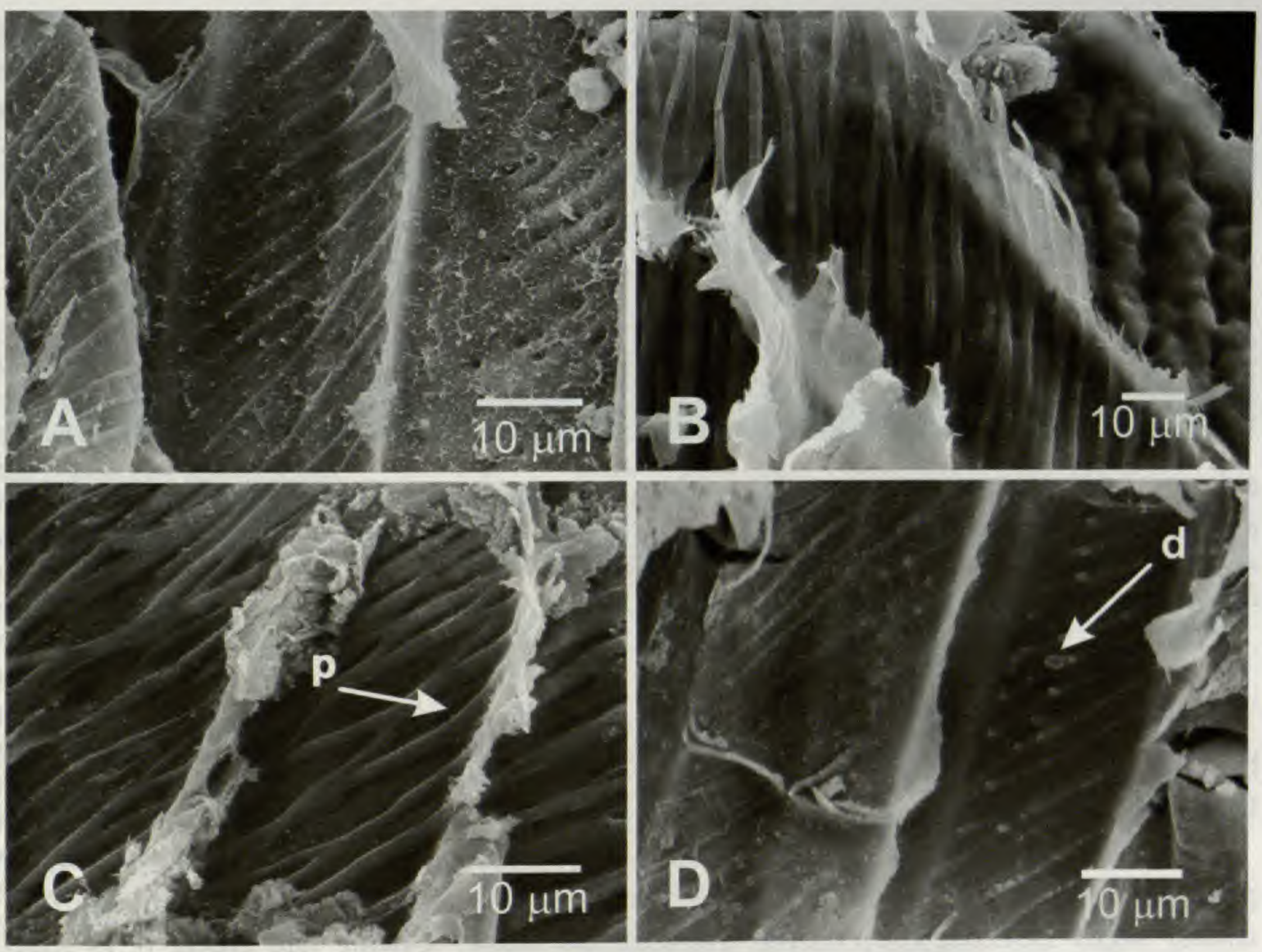

FIG. 4. Detail of the cells from the outer cortex containing thickenings observed under SEM. Thickening types. (A) Simple and furcate; (B) Furcate and anastomosing; (C) Anastomosing, among strands there are primary field pits (p); (D) Detail of the cell wall showing irregular deposits of cellulose on the thickenings (d). (A, D) *Campyloneurum aglaolepis*; (B, C) *Pleopeltis tweediana*.

resembling those of *Microgramma*. *Phlebodium areolatum* (also epiphytic) has thin thickenings (about 1.0 µm wide) and the biggest amount of thickenings per cell. The species of *Pleopeltis* (*P. hirsutissima* (Fig. 3B), *P. bryopoda* (Fig. 3C), *P. macrocarpa*, *P. minima* (Fig. 3D), *P. pinnatifida*, *P. pleopeltidis*, and *P. tweediana*), all of which are epiphytic, had the largest thickenings measurements, from 0.9–4.4 µm wide (Table 2).

Polypodium chrysolepis and *Serpocaulon gilliesii* showed similar measurements between them in relation to the width and number of thickenings per cell.

In general the number of thickenings per cell varies from 15–25 for most of the species studied. *Campyloneurum lorentzii* and *Phlebodium areolatum*, which are primarily epiphytic or rarely rupicolous, have about 30 thickenings per cell.

Most of the species studied had thickenings that were simple or furcate (Table 2). *Pleopeltis bryopoda*, a saxicolous fern, has more furcate thickenings than simple ones, and there are several per cell. Some of the furcate thickenings joined each other, but did not form a net or reticulum. This kind of anastomosing is present in 50% of the analyzed taxa (Fig. 4A–C).

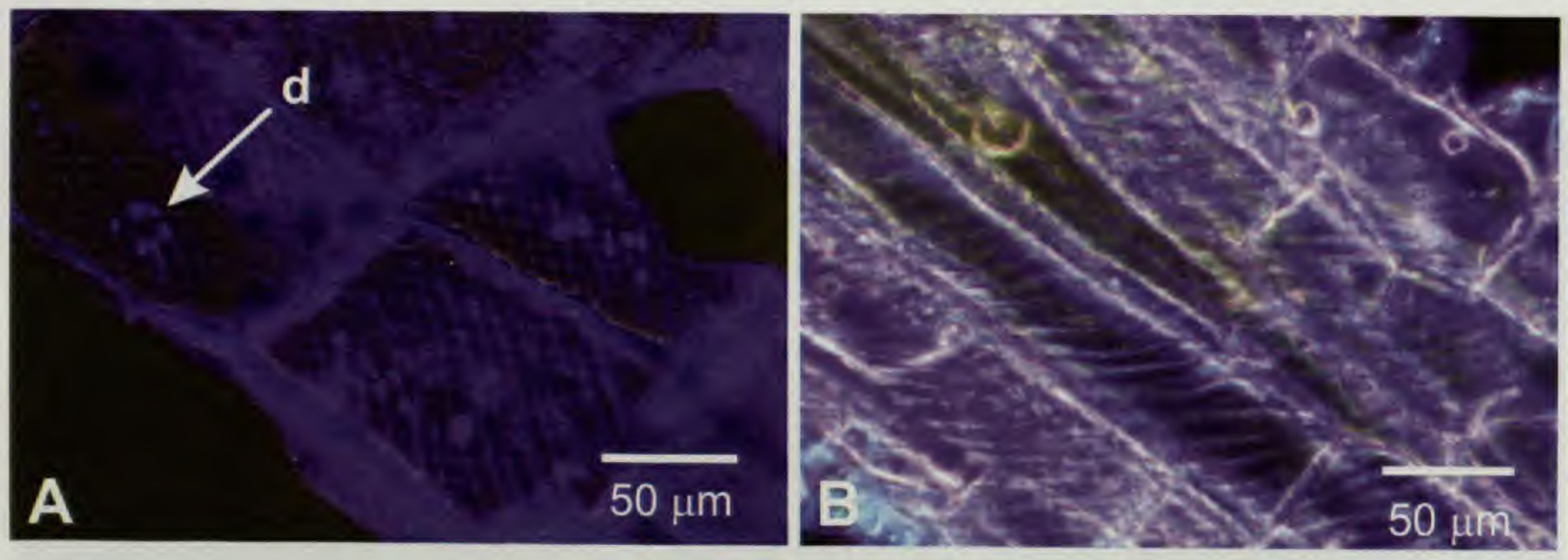

FIG. 5. Details of the cells from the outer cortex containing thickenings observed under Fluorescence Microscope using calcofluor. (A) Cellulosic thickenings of *Pleopeltis macrocarpa* with spherical deposits on the thickenings (d); (B) Positive reaction of the cellulosic thickenings of *Pleopeltis hirsutissima*.

The cells with thickenings have primary pit fields in the primary cell walls between the thickenings (Fig. 4C). Pits do not occur on the secondary wall of the thickenings.

The deposition of cellulose on these thickenings is not uniform, and it is possible to observe some rounded deposits of cellulose in certain regions under light microscope, using transmitted light. Under the SEM it is also possible to observe these rounded accumulations of cellulose on the thickenings (Figs. 4D, 5A).

The results of the staining tests revealed that the thickenings are composed mainly of cellulose. This was most evident by a positive reaction to the test with calcofluor and posterior observation under the fluorescence microscope (Fig. 5B). All tests for lignin were negative. Applying the Phloroglucinol/HCl test and the Safranine-Astra Blue double stains, the same result was also observed, the sclerenchymatic tissue situated in the inner cortex also lack lignin in their secondary walls.

Among all studied species, *Melpomene peruviana* is uniquely without thickenings in the outer cortex. But, in the other aspects its roots are morphologically similar to all investigated species in this paper. Its inner cortex has a sclerenchymatic zone and the outer cortex has cells uniformly thickened, but not forming the helical arrangement of cellulose. Initially we though that the absence of thickenings in this species could be related to its saxicolous habit, but another saxicolous species, *Pleopeltis bryopoda*, that grows together with *M. peruviana* has outer cortex with thickenings. Thus, the data suggest that there is no known correlation between the habit vs. absence of thickenings.

The results showed that the widest thickenings are found in the species of *Pleopeltis*, more specifically, in *P. hirsutissima*, that has the thickenings varying from 1.0 to 4.4 μm width and the narrowest ones appear in *Lellingeria tunguraguae* (0.5–1.2 μm) (Table 2). Both *P. hirsutissima* and *L. tunguraguae*

TABLE 2. Measurements and characteristics of the HCWT for each studied species. Minimum, average, and maximum values, plus the standard deviation and type of thickenings present in each species (S=simple; F=furcate; A=anastomosing).

Species	Helical thickenings	Width of the thickenings (μm)		Number of thickenings per cell		S	F	A
Campyloneurum aglaolepis	+	0.6(1.1)2.1	0.4	13(23)35	2.3	+	+	−
Campyloneurum lorentzii	+	0.6(1.1)1.8	0.2	17(28)40	5.3	+	+	−
Campyloneurum tucumanense	+	0.6(1.2)2.3	0.3	9(25)45	8.9	+	+	+
Lellingeria tunguraguae	+	0.5(0.7)1.2	0.2	12(15)21	3.7	+	+	−
Melpomene peruviana	−	−	−	−	−	−	−	−
Microgramma mortoniana	+	0.7(1.5)2.4	0.1	13(19)28	1.2	+	+	+
Microgramma squamulosa	+	0.6(1.3)2.9	0.4	12(18)28	1.5	+	+	+
Pecluma barituensis	+	0.8(1.2)2.4	0.4	16(21)29	2.1	+	+	−
Pecluma filicula	+	0.8(1.3)2.2	0.1	9(15)21	2.2	+	+	−
Pecluma oranensis	+	0.8(1.3)1.9	0.1	15(19)28	1.6	+	+	−
Pecluma venturii	+	0.9(1.4)2.2	0.1	11(22)35	3.5	+	+	−
Phlebodium areolatum	+	0.6(1.0)1.5	0.2	18(29)36	2.7	+	+	+
Pleopeltis bryopoda	+	0.9(2.1)4.1	0.4	8(15)23	2.8	+	+*	−
Pleopeltis hirsutissima	+	2.4(3.3)4.4	0.2	13(18)24	0.2	+	+	+
Pleopeltis macrocarpa	+	0.9(2.0)3.8	0.4	10(20)26	2.7	+	+	+
Pleopeltis minima	+	1.0(2.0)3.3	0.3	11(16)26	0.8	+	+	+
Pleopeltis pinnatifida	+	0.9(1.8)2.9	0.3	10(18)27	3.5	+	+	+
Pleopeltis pleopeltidis	+	1.7(2.3)4.0	0.2	14(21)32	3.9	+	+	+*
Pleopeltis tweediana	+	1.3(2.1)2.9	0.3	9(17)23	3.3	+	+	+
Polypodium chrysolepis	+	0.6(1.0)1.4	0.1	13(19)29	2.6	+	+	−
Serpocaulon gilliesii	+	0.6(1.1)1.7	0.2	12(20)29	3.6	+	+	−

* Represents the main type.

are epiphytes and apparently there is no correlation between the width of the thickenings and the plant habit.

The widest thickenings per cell can be found in the epiphyte species, but not all epiphytes have thickenings bigger than the rupicolous species (Table 2).

The largest number of thickenings per cell appeared in *Phlebodium areolatum* (18–36), and this species has plants with leaves up to 1 m long. However, other species show different data independent of the plant size. For example, *Campyloneurum tucumanense* has fronds up to 1 m long and yet has a widely variable number of thickenings per cell (9–45), as does *C. lorentzii* with a frond length of 85 cm long with a similar range of length variation (9–40). These data suggest that there is no correlation between the frond length and the number of thickenings on the cell walls.

DISCUSSION

According to Leroux *et al.* (2011), the presence of thickenings is not correlated with the habitat of the Aspleniaceae species. Our data for Polypodiaceae also showed that the number of thickenings per cell, and their width, are not related to the habit, habitat, or plant size.

On the other hand, Leroux *et al.* (2011) demonstrated that thickenings support a monophyletic (BS 100%) group in *Asplenium*. Unfortunately, our data for Polypodiaceae are preliminary to conclude any relation among the groups into the family based on the thickenings.

Because the regions of contact among the furcate thickenings are few (Figs. 4A, D), we called these thickenings 'anastomosing' (Figs. 4B, C) and not reticulate. On the other hand, the main kinds of thickenings observed were simple or furcated (Figs. 4A, D). Only in the epiphytic species of *Microgramma*, *Phlebodium*, and *Pleopeltis* are the anastomosing thickenings predominant; in *Campyloneurum*, another genus with epiphyte species, these anastomosing thickenings are few. According to Pandé (1935) and Ogura (1972), the thickenings in Polypodiaceae are helical and simple, whereas Leroux *et al.* (2011) classified the thickenings of Aspleniaceae into three types: simple, furcate, and reticulate.

Our results demonstrate the absence of pits in the thickenings. Leroux *et al.* (2011) also reported this for the Aspleniaceae. In the Polypodiaceae Pandé (1935) cited the presence of primary pit fields only in the cell wall between the thickenings.

About the chemical composition of secondary cell wall of roots, our results for different stains, fluorescence, and Phloroglucinol/HCl test revealed that the thickenings are basically formed of cellulose and that there is no deposition of lignin on them. Leroux *et al.* (2011) pointed out the same conclusions for Aspleniaceae. Additionally these authors commented that the thickenings present in the cell wall of the external cortical root zone are related to the mechanical protection of these small roots, acting as skeleton to the delicate roots of Aspleniaceae species to avoid root collapse during the dry periods in terrestrial, rupicolous, and epiphyte plants. The same interpretation might be applied to the species of Polypodiaceae here investigated. Among the analyzed species, all species of *Pleopeltis* (that are mainly epiphytic) showed pronounced development of the thickenings, probably as an adaptation to their revivescent ecology.

Given our results and those previously published (Schneider, 1997; Leroux *et al.*, 2011), thickenings are known only for Eupolypods I (in Polypodiaceae), Eupolypods II (in Aspleniaceae) (Smith *et al.*, 2006), respectively. Therefore, this character might be better explored for all Polypodiaceae members. It is highly probable that these structures appear in other members of Polypodiales, but more studies are needed to confirm this hypothesis. In fact, the presence of this structure in other groups of ferns, their types, and its evolution into ferns and lycophytes remains obscure.

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Shorter Notes

Hydrochemical Characterization of A Stand of the Threatened Endemic *Isoëtes malinverniana*

Isoëtes malinverniana.—*Isoëtes malinverniana* Ces. & De Not. is an aquatic quillwort endemic to Northern Italy that, as with many other quillworts, is facing drastic changes in its habitat (Wen *et al.*, J. Freshwater Ecol. 18:361–367. 2003.). *Isoëtes malinverniana* grows in running water canals used for rice field water supply (usually with *Ranunculion fluitantis* vegetation), but in the past it probably occurred in natural streams generated by springs and minor river branches, characterized by oligotrophic waters (Abeli *et al.*, Aquatic Conserv: Mar. Freshw. Ecosyst. 22:66–73. 2012). During the last forty years the distribution range of *I. malinverniana* has been rapidly decreasing as a consequence of changes in the rice cultivation practices in Northern Italy. Particularly, the use of herbicides and fertilizers, the regimentation of water courses with water removal in winter, and the mechanical re-profiling of the canals are the major threats to the species (Barni *et al.*, Aquat. Bot. 107:39–46. 2013). Although the species is protected at European and national levels, its conservation status is critical (Bilz *et al.*, European Red List of Vascular Plants. Publications Office of the European Union, Luxembourg. 2011; Rossi *et al.*, Lista Rossa della Flora italiana. 1. Policy Species e altre specie minacciate. Comitato Italiano IUCN e MATTM. 2013), and urgent conservation actions are needed to stop the decline of the species, by reinforcing some extant populations and/or reintroducing new populations within the historical range. The major problem with respect to reintroduction actions is the fact that the original habitat of *I. malinverniana* has been greatly modified. This reduces the probability of successful translocations and also affects the possibility to study the real ecological requirements of the species. However, a site of *I. malinverniana* with about 30 plants discovered a few years ago in a natural stream in the Ticino river basin at La Sforzesca near Vigevano (voucher specimen in PAV) still has many of the characteristics of the original habitat. Here we have analyzed the ion concentrations of surface water and, for the first time, sandy sediment pore water along a 30 m long transect crossing the population. Pore water samples were collected in three points along the transect at about 10 m from each other, while a single surface water sample was collected in the middle of the transect. Sediment pore water samples were collected at a depth of about 10 cm using ceramic cups (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands), connected to 100% vacuum PVC syringes (50 ml) by means of a PVC tube, according to Van Der Welle *et al.* (Freshwater Biol. 52:434–447. 2007). For each water sample, pH, electrical conductivity and temperature were immediately recorded, while ion concentrations were analyzed at the laboratory of the Radboud University (Nijmegen, The Netherlands).

Surface water pH, electrical conductivity and temperature were identical among the three samples (6.8; 271 µS/cm; 11°C, respectively). This pH was

lower than in populations within channels used for rice field water supply (Barni *et al.*, 2013; Table 1), but higher than the mean pH values found in Dutch and Norwegian populations of *I. lacustris* and *I. echinospora* (Table 1). The pH of the sediment pore water was remarkably high, reaching a mean value of 8.7 ± 0.9, which also results in very low pore water carbon dioxide concentrations. Mean pH values in Dutch and Norwegian *Isoëtes* stands are around 6, whereas pore water carbon dioxide concentrations are typically (much) higher than surface water concentrations (Table 1). Being able to take up carbon dioxide by the roots, this situation provides a competitive advantage for isoëtid species compared to non-isoëtid species, which lack this ability (Smolders *et al.*, Aquat. Bot. 73:325–350. 2002). Therefore *Isoëtes* species thrive very well in soft water lakes and streams where carbon availability in the water layer is low and species dependent on carbon uptake from the water layer are unable to grow. The high calcium, magnesium and bicarbonate concentrations of the water layer and sediment pore water indicate that the water in the stand from the Ticino river basin is relatively well-buffered compared to the Dutch and the Norwegian *Isoëtes* stands, which were found in weakly buffered soft waters. The Ticino population is probably dependent on the uptake of carbon dioxide from the water layer where carbon dioxide concentrations are much higher than in the sediment pore water. Pedersen *et al.* (J. Exp. Bot. 62:4691–4700. 2011.) showed the potential gas exchange via the leaves to be substantial for *I. australis*, although the resistance to gas exchange was up to three times higher than for roots. The uptake of CO_2 via the roots may have further lowered CO_2 concentrations and indirectly increased the pH of the sediment pore water in the Ticino stand.

Regarding the surface water, the concentration of phosphate, total-P and ammonia are at the lower end of the ranges found for Dutch and SW Norwegian *Isoëtes* stands and are also lower than the mean values found for other *I. malinverniana* stands (Table 1). However, the nitrate concentration is unnaturally high (Table 1), suggesting water nutrient enrichment probably due to the presence of rice and cornfields just a few hundred meters upstream from the population.

High concentration of nitrate was also evident in sediment pore water. Although for the parameters analyzed in the sediment pore water, it was not to possible to make a comparison with other stands of *I. malinverniana*, pore water nitrate was very high compared with values measured in Dutch and Norwegian *Isoëtes* stands (Table 1) and values reported for isoëtid lakes in Spain (Catalan *et al.*, Hydrobiologia 274:17–27. 1994; Gacia *et al.*, J. Limnol. 68:25–36. 2009) and in Scandinavia (Vestergaard & Sand-Jensen, Aquat. Bot. 67:85–107. 2000). *Isoëtes* species generally grow on mineral, usually sandy, sediments with low oxygen consumption rates and actively maintain the sediment in an oxidized state by leaking oxygen via the roots (Pedersen *et al.*, 2011). Due to the oxidized conditions nitrate, iron and sulphate reduction, are normally not important in isoëtid stands. This results in low iron concentrations in sediment pore water, as iron is not mobilized by iron reduction (Table 1), which also results in a low mobility of phosphorus, which is

TABLE 1. Ion concentration for surface water and sediment pore water of a stand of *Isoëtes malinverniana*. Values of surface water concentration from Barni *et al.*, 2013 and unpublished data for three *Isoëtes* stands from the Netherlands (A.J.P. Smolders) and different *Isoëtes* stands from 9 lakes in SW Norway, sampled in 1995 and 2010 (E.C.H.E.T. Lucassen) are shown for comparison. Data are expressed in µmol/L.

	Barni et al. (2013)	This study			
Surface water	*Isoëtes malinverniana*		(Neth, 2005) *Isoëtes*	(Norw, 1995) *Isoëtes*	(Norw, 2010) *Isoëtes*
pH	7,6	See text	5,8 (0,2)	5,7 (0,7)	6,2 (0,6)
HCO3		1303	35,3 (7,5)	12,0 (25,5)	113 (168)
CO2		454	123,3 (13,7)	76,4 (16,3)	84,1 (88,8)
P		0,4	3,0 (1,9)	0,8 (0,7)	0,4 (0,3)
PO4	3,55	0,13	0,4 (0,2)	0,3 (0,3)	0,3 (0,3)
NO3	26,6	149	16,1 (13,7)	11,1 (5,1)	9,2 (7,2)
NH4	5,5	1,6	12,7 (8,3)	3,8 (3,4)	2,7 (3,7)
Ca	520	819	246 (145)	38,3 (20,3)	35,9 (16,1)
Mg		277	81,5 (36,5)	23,8 (6,2)	23,5 (5,0)
Na		316	413 (191)	145 (41,0)	182 (25,0)
Cl		216	613 (290)	166 (41,6)	172 (28,5)
K		54,4	110 (57,8)	10,4 (11,0)	13,8 (13,0)
Fe		0,1	6,5 (5,9)	1,2 (0,9)	1,8 (2,2)
Mn		0,02	2,2 (2.3)	0,2 (0,2)	0,1 (0,1)
SO4		302	107 (35,5)	31,9 (8,0)	22,6 (1,9)
Si		181	36,6 (30,8)	15,7 (5,8)	6,2 (7,4)
Al		0,02	13,1 (8,6)	7,3 (3,2)	1,8 (0,6)
n		1	3	8	9

	Barni et al. (2013)	This study			
Pore water		*Isoëtes malinverniana*	(Neth, 2005) *Isoëtes*	(Norw, 1995) *Isoëtes*	(Norw, 2010) *Isoëtes*
pH		8,7 (0,9)	6,0 (0,1)	5,9 (1,0)	6,1 (0,5)
HCO3		3069 (569)	395 (189)	175 (224)	192 (175)
CO2		16 (3)	730 (115)	336 (306)	275 (177)
P		9,6 (1,1)	3,1 (2,3)	0,6 (0,4)	1,5 (0,8)
NO3		119 (21,9)	10,3 (12,6)	11,8 (12,7)	15,5 (17,1)
NH4		3,5 (3,4)	29,4 (13,6)	3,9 (2,2)	1,7 (2,2)
Ca		1021 (95)	337 (169)	102 (61,4)	146 (127)
Mg		816 (326)	126 (54,7)	73,8 (55,5)	60,8 (34,8)
Na		1988 (802)	435 (192)	214 (78)	228 (122)
Cl		1780 (643)	435 (192)	192 (94)	197 (121)
K		330 (186)	96,7 (31,4)	13,7 (19,3)	31,0 (25,0)
Fe		0,2 (0,2)	8,1 (4,9)	2,6 (2,2)	0,9 (1,3)
Mn		1,3 (1,6)	3,1 (0,8)	1,2 (1,8)	0,4 (0,7)
SO4		344 (16,4)	200 (233)	70,9 (51,6)	69,4 (17,4)
Si		246 (25,5)	83,5 (55,6)	98,3 (68,7)	125,9 (90,7)
Al		0,2 (0,1)	21,9 (18,2)	10,3 (7,7)	2,9 (6,6)
n		3	3	30	20

efficiently bound to oxidized iron(hydr)oxides (Smolders *et al.*, 2002). Also the observation that at our location nitrate and sulphate concentrations do not differ between water layer and pore water suggest the lack of microbiological consumption of nitrate or sulphate in the sediment, indicating oxidative conditions in the sediment.

The chemical characterization of the *I. malinverniana* habitat shows that the species is growing on an oxidized sediment with a relatively low availability of phosphorus. The water layer and sediment are relatively well buffered and characterized by a high pH and a very low availability of carbon dioxide in the sediment pore water. Although the surface water phosphorus concentrations are still low, the nitrate enrichment due to the intensive agricultural activity of the area is evident even in a site apparently less impacted than other stands. As a consequence even a temporary increase of the phosphorus availability in the water layer might easily lead to an excessive growth of algae. This poses serious threats for the conservation of this endemic species for which a suitable site for translocation is at the moment unavailable. Flowing water may strongly benefit the species under more eutrophic conditions because in flowing water algae are less likely to become dominant and uptake of CO_2 from the water layer is facilitated. Nevertheless increased nutrient levels in the water layer will at least lead to the growth of epiphytic algae as has been observed in many of the remaining *I. malinverniana* populations (e.g. Arborio, Vigevano), which may depress growth by shading and by depletion of inorganic carbon and nutrients at the leaf surface.—T. Abeli (e-mail: thomas.abeli.it@gmail.com), S. Orsenigo and N. M. G. Ardenghi, DSTA, Department of Earth and Environmental Sciences, University of Pavia, via S. Epifanio 14, 27100, Pavia, Italy, E.C.H.E.T. Lucassen and A.J.P. Smolders, Department of Aquatic Ecology and Environmental Biology, Radboud University, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands.

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Review

Flora of China, vols. 2–3, Lycopodiaceae through Polypodiaceae, by Wu Zhengyi† and Peter H. Raven, Co-chairs of the Editorial Committee and Hong Deyuan, Vice Co-chair of the Editorial Committee. Science Press (Beijing) and Missouri Botanical Garden Press (St. Louis). ISBN 978-0-915279-34-0 (series), ISBN 978-1-935641-11-7 (vols. 2–3). Publication date: 6 June 2013.

959 pages; English, with Chinese authors' names and Chinese common names in both roman and Pinyin, and roman and Pinyin indices of Chinese common names (no common names are given in English).

No standard format for citing this work is suggested, but the MBG Press website indicates that the author of the series is the Flora of China Editorial Committee.

Available from the Missouri Botanical Garden Press (www.mbgpress.info). $190 plus shipping. Text also available on-line at www.efloras.org.

The final text volume of the great new Flora of China, published as a cooperative venture by a consortium of Chinese botanists and international experts, has arrived. Inspired by Peter Raven, it was originally proposed at a joint meeting of Chinese and American botanists held at the University of California, Berkeley, in 1979. It was formally initiated in 1988 with the appointment of a joint Sino-American Editorial Committee, with Raven and Wu as Co-chairs. Further details of its inception are given in the Foreword to the first published volume, 17: vii–viii, 1994. It is a testament to the foresight of Raven and Wu (who passed away in June 2013), and a proud achievement for China and the world botanical community. Planned as an updated and revised English language edition of the Flora Reipublicae Popularis Sinicae (FRPS, 1959–2004), it is remarkable that this Flora – now 24 volumes bound in 21 books and awaiting only the introductory volume – has been brought to fruition in such a short time. Just coordinating the contributions from 59 authors for the current volume (26 Chinese authors and 33 others) must have been a difficult task. The work was undertaken at eleven botanical institutions, four in China and seven elsewhere, with overall organization and coordination at the Missouri Botanical Garden. Details may be seen at the Project website, flora.huh.harvard.edu/china. Throughout the entire Flora, the Editorial Committee has been under the unbroken and steady leadership of both Raven and Wu. The undertaking was massive, its execution nearly flawless. This combined volume is dedicated to the Chinese pteridologist Ching Ren-Chang (1898-1986), who was Secretary General of the FRPS project.

For pteridologists, last is best, given the substantial progress made in our understanding of the phylogenetic relationships of ferns and lycophytes since the project began, not to mention the additional floristic knowledge and discoveries. We commend the editors and authors, and recommend the book to anyone seriously interested in Chinese botany or ferns.

China is enviably wealthy in pteridophytes, particularly ferns, and this wealth is arrayed for study by anyone who takes up this book. As stated in the preface, "[The pteridophyte flora of China] includes 38 families, 177 genera, and 2,129 species, among which three genera and 842 species are endemic to China, and one genus and four species are introduced to China." This is eye opening for North American fern lovers: we have only 96 genera and 554 species (FNA 1993). Various historical events, especially the Himalayan orogeny and the recent glaciations of North America, have set the two areas on different paths, and China is the richer by far. One has only to contemplate its 123 species of *Athyrium* vs. our two species, its 167 species of *Dryopteris* to our fourteen, or its 207 species of *Polystichum* to our fifteen (we share four), to wonder how different things could have been. Our two regions share approximately 60 indigenous species but for the most part, these do not occur in the classic East Asia – Eastern North America pattern so well known in angiosperms. Instead, most of our shared species are circum-north temperate or circumboreal, e.g., several lycopods, eight horsetails, and such species as *Cryptogramma stelleri*, *Woodsia ilvensis*, *W. glabella*, *Gymnocarpium jessoense*, and *Dryopteris fragrans*.

The volume has only short prefatory remarks, without discussions of geography, climate, or floristic associations. The heading of the main text is labeled "Pteridophytes (Lycophytes and Ferns)" which, for ferns, transitions away from the recently popular "monilophytes." Families are presented in taxonomic order, based on the linear classification of Christenhusz et al. (2011), itself based largely upon Smith et al., (2006). Following Christenhusz et al., Equisetaceae are treated first (vs. Ophioglossaceae in the scheme of Smith et al. 2006), as sister to the rest of the ferns.

Treatments are in standard format and include dichotomous keys and full descriptions of families, genera, and species. Subspecies, varieties and, in a very few instances, forms are treated where authors deem necessary. Hybrids are mentioned, at least in some genera, but are not treated in depth. Keys and descriptions show the high standards that one anticipates from previous volumes. The descriptions are extremely detailed, most involving 75–100 morphological characters and often running from 25 to 35 or even 40 lines, although the treatments of Hymenophyllaceae are notably shorter. Short or full synonymies are given, but types are not reported. Each species description is followed by a statement of habitat, a list of provinces (including Taiwan and islands of the South China Sea) where it occurs, and a statement of worldwide range. Following that are notes about relationships, variability, medicinal uses (if any), and nomenclatural problems. These notes offer insight into historical and current taxonomic concepts, problems in the flora, and often additional comparative details of identification. They will be of great interest to current and future pteridologists as our knowledge improves and will no doubt prompt studies for many years to come.

Inevitably, some families (e.g., Ophioglossaceae) lack recent research focused on China, while others (e.g., Aspleniaceae) benefit from recent and ongoing scrutiny, so the treatments are slightly uneven by subject, but they

consistently include the best research available. More thoroughly than many floristic treatments, attention is paid to enumerating (if not explicating) taxa about which little is known, or for which types or other supporting specimens have not been seen. Thus, many genera are followed by lists of "uncertain taxa." Such careful explication of the lack of knowledge can be very helpful and is much better for future users than simply glossing over such gaps.

There will always be areas of taxonomic disagreement, and such disagreements can make for good discussions. Fortunately in most cases, justification of the author's choices is given, either directly or by reference to literature. Notably, all references are given in text rather than in a separate section. We think the following taxonomic decisions may enliven the discourse:

- The recognition of various generic segregates and species assignments in Lycopodiaceae, especially the recalcitrant *Lycopodium sensu medio*, in which North American plants are now rather standardly treated in several segregate genera i.e. *Palinhaea*, *Diphasiastrum*, *Dendrolycopodium* (not mentioned in synonymy), and *Spinulum*.
- The inclusion of *Pseudolycopodiella* in *Lycopodiella*. We suspect that debate over generic limits in Lycopodiaceae will continue for the foreseeable future.
- The presence of *Huperzia appressa* (type from Newfoundland) and *H. lucidula* (type from eastern North America) in China. *Huperzia appressa* in particular has not frequently been recognized outside North America.
- That *Adiantum aleuticum* is subsumed into *A. pedatum* (type from eastern North America). This disposition neglects evidence that the two taxa are reproductively isolated (Paris 1998). Perhaps the *Adiantum* authors' decision was based on the results of Lu et al. (2012) who, using chloroplast markers, showed that *A. aleuticum* is nested within *A. pedatum*. Even so, Lu et al. (2012) concluded that "Chinese *A. pedatum*, the Japanese *A. pedatum* ... and the eastern North American *A. pedatum* are not the same taxon."
- The recognition of *Cystopteris dickieana* in China, albeit with the caveat that Scottish (i.e., typical) and North American plants "are considered conspecific with *C. fragilis*." The authors' phrase, "the taxonomic status of Chinese *dickieana* (i.e., plants with rugose or verrucose perispores) is uncertain" serves to highlight that this is still an unresolved problem. Perhaps the Chinese plants should not have been given species status at this juncture. No doubt cryptic species are hiding in *C. fragilis* in China, as elsewhere.
- Placement of the contentious genus *Cystoathyrium* in Cystopteridaceae. *Cystoathyrium* comprises a single, rare, and perhaps extirpated species endemic to forested areas in western Sichuan. Authors Zhongren and Kato include it in Cystopteridaceae with text contrasting it to both *Cystopteris* and *Athyrium*. We agree because, although homoplastic within Eupolypods II, the character states exhibited by *Cystoathyrium* occur more frequently in Cystopteridaceae than in Athyriaceae.

- The treatment of both *Asplenium dolomoticum* and *A. quadrivalens* as species within the *A. trichomanes* complex. Historically, this complex has been important in fern systematics in terms of shifting botanical science from descriptions to explanations, and so it continues to provide food for thought.
- *Asplenium komarovii* is the name used for tetraploid plants of A. *scolopendrium*. Whether or not this is equivalent to the North American tetraploid *A. scolopendrium* var. *americanum*, listed as threatened under the federal Endangered Species Act, will be of interest.
- The adoption of many new generic concepts in Polypodiaceae among both grammitids and polypods. We expect this to facilitate recognition of taxa since the new groups are morphologically more cohesive than previous classifications. These concepts match those used in the recently published Flora of Peninsular Malaysia, Series I: Ferns and Lycophytes, Volumes one and two (Parris et al., 2010, 2013), itself a valuable supplement to the current volumes for more tropical elements of the Chinese flora. The generic concepts are also matched for the most part by Zhang's (2012) recent photographic guide to ferns of China, although Zhang retained *Oreogrammitis* and *Radiogrammitis* in *Grammitis*.
- The sinking of *Dryopteris* subgenus *Nothoperanema*, *Dryopteris* subgenus *Erythrovariae* and *Dryopteris* section *Dryopsis* into *Dryopteris*, a decision that places *Acrophorus*, *Diacalpe*, *Dryopsis*, *Nothoperanema*, and *Peranema* into synonymy. Evidence for this dates back to the phylogenies presented by Geiger and Ranker (2005), Li and Lu (2006), and Liu et al. (2007), but combinations (Zhang and Zhang, 2013; Zhang et al. 2013 a) were not made until publication of the comprehensive study by Zhang et al. (2013c).

More than 150 taxonomic innovations are presented and seven new species (in *Lycopodium*, *Asplenium*, *Polystichum*, *Radiogrammitis*, and *Oreogrammitis*) and two new nothospecies (in *Asplenium*) are described. Most of the others are transfers including 41 to *Deparia* (many previously in *Dryathyrium*), 32 to *Selliguea* (mostly previous *Phymatopteris*), and 23 to *Diplazium*. Besides those in normal sequence, a few new combinations are buried in text and could be easily overlooked, e.g., *Hymenasplenium rivulare* (Fraser-Jenkins) Viane & S. Y. Dong, made in the discussion of the genus, and *H. ikenoi* (Makino) Viane, made under the discussion of *H. cardiophyllum*. Fortunately, a complete list of novelties is presented in the back to aid indexers.

A feature of this combined volume is a glossary specific to the subject matter, in this instance adapted from Lellinger (2002). (A similar glossary is also included in vol. 22, Poaceae. Unfortunately, at least two errors are here, there being no definition of "scale" (implied to be present by reference under "costate") and no definition of "pteridophytes" (implied to be present by reference under "ferns"). One might also quibble that use of the word "gemma" for the bulbils of some *Huperzia* is not attributed to North American literature, e.g., FNA (1993) as well as European and African literature. Nor is "gemma," as defined in the glossary ("a structurally specialized asexual propagule found on some gametophytes...") mentioned in the treatments of Hymenophyllaceae, where it would be expected.

Some of the text, perhaps inevitably, leaves the glossary aside so that there is occasional use of non-standard terminology. For example, *Ceratopteris* is described as "juicy," *Diplaziopsis* as having "semi-circular" spores, Rhachidosoraceae as having "2-sided spores with flakelike folds in the perispore" and *Cystoathyrium* as having a stipe base that is not "beak-shaped." "Bract," not defined in the glossary, is used to mean a microphyll on the peduncle in *Lycopodium* and *Lycopodiella* but in Glecheniaceae refers to the bud-covering structures elsewhere called "pseudostipules" (Shaw and Ranker 2011).

Typographic errors are virtually or actually nonexistent, as we have found none to date. The misspelling of *Parathelypteris noveboracensis* as *novoboracensis* (p. 327, in discussion of *Parathelypteris nipponica*) is repeated in the index so it is not merely a typo. We noted only a very few factual errors on first study: *Lycopodiastrum* is described as a woody vine when in fact all lycophytes are non-woody plants, merely stiff and lignified. North America is not included in the range of *Parathelypteris*. The term acrostichoid appears in the glossary, but is not employed in descriptions where it should be in *Dryopteris* subgenus *Elaphoglossoideae*, while in *Bolbitis*, where it is applied, the sori are superfluously described as exindusiate (superfluous because this is implied in the definition of acrostichoid). *Bolbitis* is further said to have spores with a thick epispore, a term normally applied to spores of *Equisetum* and the heterosporous water ferns; perhaps perispore was intended. Finally, the term homomorphic, as used in the key to the Tectariaceae, is misapplied and should be replaced with monomorphic.

The Flora of China will be hard to surpass in this century, at least in a book format. Field guides and local floristic efforts – many of which will be web-based – may be more colorful and may present much additional information, but this volume will stand for many years as the authoritative resource on the lycophytes and ferns of China. Even more, its publication will inform and enliven pteridophyte research throughout Asia, as participants affirmed at the 2013 Flora Malesiana conference in Bogor, Indonesia, even as they anticipated taxonomic attention shifting deeper into the tropics. Moreover, it will shift the focus from taxonomic to evolutionary questions: speciation, breeding systems, and reticulate evolution remain largely unexplored in the region. We very much look forward to the introductory volume for perspectives on ecology, and to the soon forthcoming companion volume(s) of illustrations.

In 2006, one of us (MS) had the fortune to accompany Flora authors David Barrington, He Hai, and LiBing Zhang on a collecting trip through Sichuan and Yunnan Provinces focused on resolving issues in Dryopteridaceae. Browsing the completed treatments brings back the thrill of botanizing Mt. Omei and Yulong, and the daunting complexity of Chinese *Polystichum*. Having the full flora in hand now leaves us eager to dig deeper into the ferns and lycophytes of this fascinating region.—ARTHUR V. GILMAN, Gilman & Briggs Environmental, 1 Conti Circle, Suite 5, Barre, Vt 05641; MICHAEL A. SUNDUE, The Pringle Herbarium, Dept. of Plant Biology, Torrey Hall, 27 Colchester Ave., University of Vermont, 05405, U.S.A.

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American Fern Journal 103(4):251 (2014)

Erratum

AFJ volume 103 issue 2, pp. 112-117 (April – June 2013)

Figure 1 in the *American Fern Journal* article entitled ***Myriopteris windhamii* sp. Nov., a New Name For *Cheilanthes villosa* (Pteridaceae)** by Amanda L. Grusz was originally published incorrectly. The correct figure is shown below.

Fig. 1. Scanned image of the holotype of *Myriopteris windhamii* (*Windham 4165*, DUKE). Scale bar in centimeters.

American Fern Journal 103(4):252–254 (2014)

Erratum

AFJ volume 103 issue 3, pp. 175–181 (July–September 2013)

Figures 1 and 2 in the *American Fern Journal* article entitled **A New Hybrid of *Serpocaulon* (Polypodiaceae) from Costa Rica** by Alexander Fco. Rojas-Alvarado and José Miguel Chaves-Fallas were originally published incorrectly. The correct figures are shown on the next two pages.

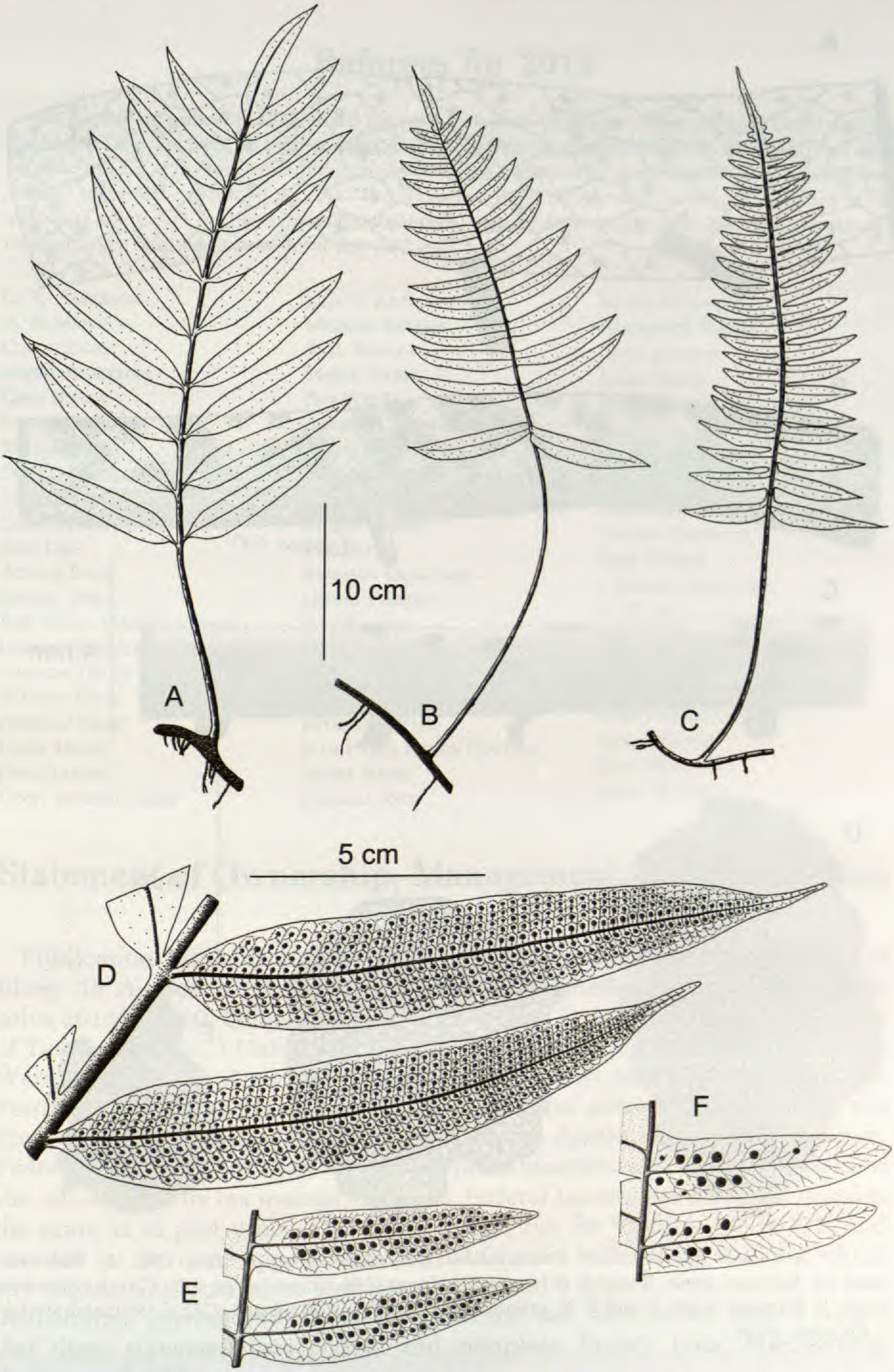

FIG. 1. A and D. *Serpocaulon fraxinifolium* (*J.M. Chaves et al. 297*, CR); A. Habit. D. Blade detail. B and E. *S.* × *sessilipinnum* (*J.M. Chaves et al. 299*, CR); B. Habit. E. Blade detail. C and F. *S. ptilorhizon* (*J.M. Chaves et al. 298*, CR); C. Habit. F. Blade detail.

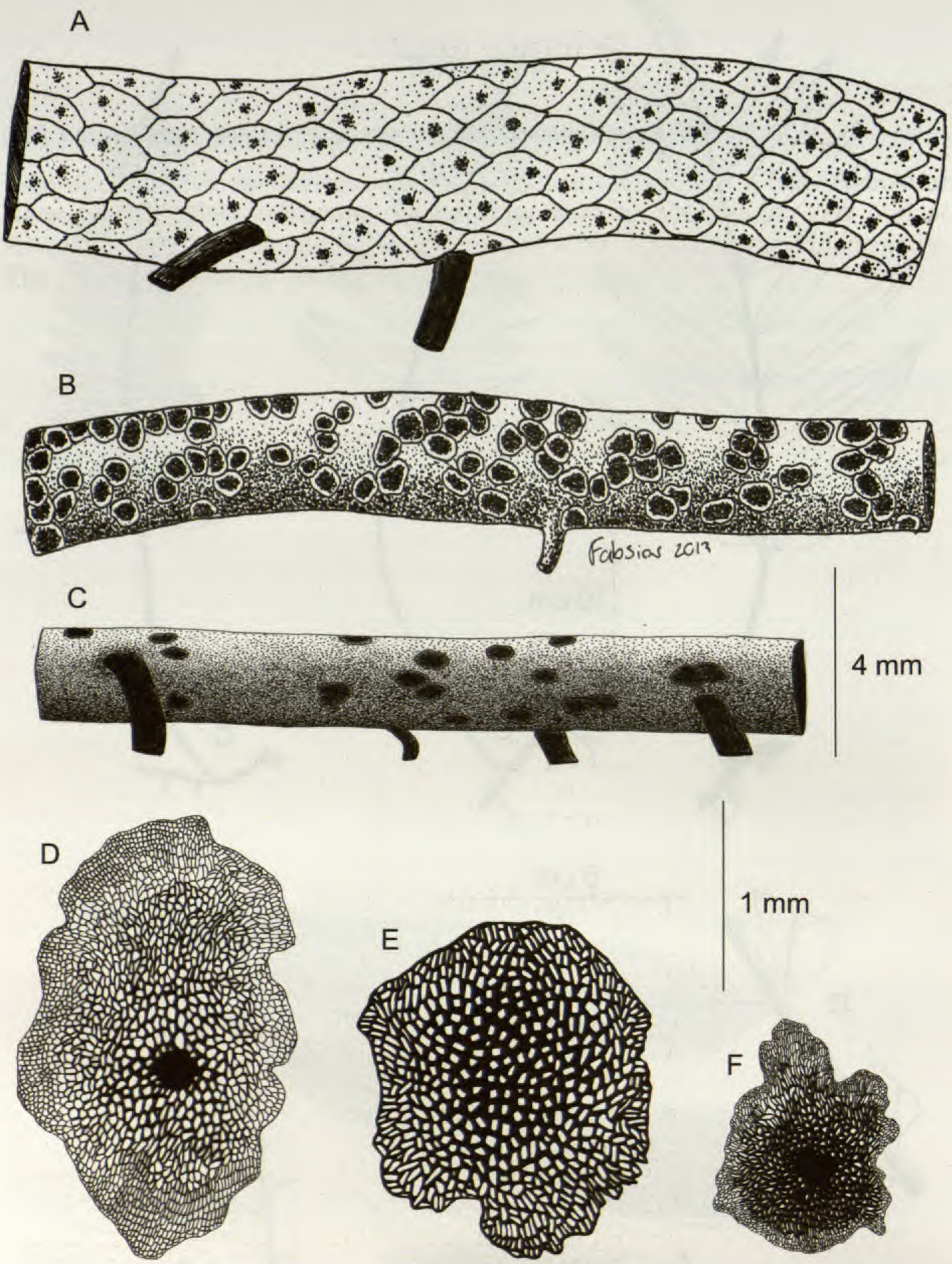

FIG. 2. A and D. *Serpocaulon fraxinifolium* (*J.M. Chaves et al. 297*, CR); A. Rhizome detail. D. Rhizome scale. B and E. *S.* × *sessilipinnum* (*J.M. Chaves et al. 299*, CR); B. Rhizome detail. E. Rhizome scale. C and F. *S. ptilorhizon* (*J.M. Chaves et al. 298*, CR); C. Rhizome detail. F. Rhizome scale.

American Fern Journal 103(4):255 (2014)

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All papers submitted to the journal are peer reviewed. Members of the editorial board and the American Fern Society, as well as additional scientists in cognate areas do these reviews on a voluntary basis. It is their work that contributes to the high quality of articles in the *American Fern Journal* and to its continued success. The American Fern Society and I extend our thanks to the following reviewers for the assistance, diligence, and patience in the year 2013 (I apologize if I inadvertently omitted anyone from this list).

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